DENITRIFICATION IN A PASTURE SOIL

GWENDOLINE MARY EGGINTON, B.Sc., M.Sc.

Thesis submitted for the Degree of Doctor of Philosophy
in the Faculty of Science, Department of Agriculture.

UNIVERSITY OF EDINBURGH

1983

DECLARATION

I, Gwendoline Mary Egginton, declare that this thesis was composed by myself, and the work described was carried out by myself.

Signed

ACKNOWLEDGEMENTS

The author wishes to express her sincere thanks to the following:

Dr. K.A. Smith of the Edinburgh School of Agriculture, Soil Science Department, for his guidance and supervision of this work, and patience and encouragement during the preparation of this thesis.

Dr. B.C. Ball of the Scottish Institute for Agricultural Engineering for advice on the measurement of gaseous diffusion coefficients.

Dr. I. Dawes of the Microbiology Department for his supervision of the investigation of acetylene utilizing bacteria.

Members of staff of the Soil Science Department, particularly Walter Macdougall and Frank Geddes for cheerful help with the field experiments often in unpleasant conditions.

The Edinburgh School of Agriculture for providing space and research facilities.

Jackie Bogie and Christine Struthers for typing this thesis.

Stephen Parish for his encouragement and help with computing.

ABSTRACT

The effect of multiple applications of cow slurry or inorganic nitrogen fertiliser (calcium nitrate) on the composition of the soil atmosphere was studied over three years, in an imperfectly drained pasture soil. Irrespective of fertiliser treatments concentrations of oxygen (O_2) decreased and carbon dioxide (CO_2) increased during the winter months, but remained near ambient levels during the summer. Slurry decreased O_2 concentrations temporarily, especially following applications in the autumn and in the spring. Concentrations of nitrous oxide (N_2O) remained close to ambient during the summer under all treatments but increased over the autumn, winter and spring, especially in the few weeks following fertiliser applications. Enhanced N_2O concentrations were significantly related to decreases in O_2.

The relative diffusivity of soil cores from the field site was determined from the rate of diffusion of ^{85}Kr through the cores. From this, the diffusion coefficient for N_2O was calculated and used to estimate the flux of N_2O from the field plot. Losses from plots receiving inorganic fertiliser were up to 6kg N ha^{-1} a^{-1} but lower than this from control plots and plots receiving slurry.

In the 3rd year acetylene (C_2H_2) was used to inhibit the reduction of N_2O to N_2 in microplots enclosed by plastic cylinders driven into the soil, so that total gaseous losses could be measured. These were low during the summer and in the winter in all treatments, but increased during the autumn and spring. Losses of N were highest in the inorganic fertiliser treatment: 10 and 21% of the N applied in the autumn and spring, respectively. Fluxes of up to 115g N $ha^{-1}h^{-1}$ were recorded. Losses from the slurried plots were only slightly higher than from the control plots. When fluxes were low, most loss of N was as N_2O, but the proportion lost as N_2 increased as the total flux increased. The highest ratio of $(N_2 + N_2O):N_2O$ recorded was 25.

In laboratory experiments C_2H_2 was found to inhibit nitrification totally at a concentration of 0.04ml ml^{-1} but the effect was found to be reversible. Acetylene also inhibited N_2O reduction. In aerobic

conditions C_2H_2 increased soil respiration, some of the C_2H_2 being consumed in the process. Several colonies of bacteria were isolated from the soil able to use C_2H_2 as their sole carbon source.

CONTENTS

LIST OF FIGURES

Page

LIST OF PLATES

1. REVIEW OF THE LITERATURE AND INTRODUCTION TO EXPERIMENTAL WORK

1.1. Introduction

The microbiology and biochemistry of denitrification is reviewed. This section includes a discussion of the effect of acetylene (C_2H_2) on denitrification and other microbial processes, since C_2H_2 was used as an inhibitor of nitrous oxide (N_2O) reduction both in field and laboratory experiments.

Since denitrification only occurs in anaerobic soil, soil aeration is discussed. Particular emphasis is laid on its assessment in the field, since this constituted a large part of the research undertaken in the project.

The methods used to study denitrification both in the laboratory and the field are considered together with the results of such studies, including those aimed at assessing the extent of nitrogen loss in the field.

Finally the work undertaken in this project is introduced.

1.2. Microbiology and Biochemistry of Denitrification

In denitrification, nitrate (NO_3^-) replaces oxygen (O_2) as the terminal electron acceptor during respiration and is reduced to oxides of nitrogen, or nitrogen (N_2), the end products being excreted (dissimilative reduction). Since the energy yield for O_2 as terminal acceptor is greater than for NO_3^- (26.5 and 18 kcal mol^{-1} electrons respectively) denitrification occurs only in the absence of O_2.

Denitrification should not be confused with assimilative reduction of NO_3^- to ammonium (NH_4^+) which occurs in green plants, algae, many fungi and some bacteria, or dissimilative NO_3^- reduction (NO_3^- respiration) where the end product is nitrite (NO_2^-).

1.2.1. Microbiology

Denitrification has long been known as a microbiological process (Gayon and Dupetit, 1882). However, in sterile soil, NO_2^- can break down to nitric oxide (NO) and N_2 (Bulla *et al.*, 1968, 1970), nitrous oxide (N_2O) and N_2 (Burford and Stefanson, 1973), and in acid soils

to NO (Allison, 1963). These processes, often referred to in the literature as chemical denitrification, are not thought to contribute greatly to gaseous losses of N in the field except under unusual conditions.

Although some fungi are known to produce some N_2O during dissimilatory NO_3^- reduction (Bollag et al., 1972a), the only true denitrifiers are bacteria. Nitrate respirers make up a small proportion of the aerobic bacterial population (1% in a study by Doner et al., 1975), and of these only a small proportion can denitrify: Volz et al. (1974) found 10% and Gamble et al. (1977) 25%.

Denitrifying bacteria occur in diverse habitats, including the oceans (Barbaree and Payne, 1967), all kinds of soil (Jordan et al., 1967; Gamble et al., 1977; Garcia, 1977a), river sediments (Hill, 1979) and lake sediments (Tiren et al., 1976; Kamp-Nielsen and Anderson, 1977). Denitrifying bacteria are also known which are plant pathogens (Pichinoty et al., 1977) and which can fix N_2 (Rigaud et al., 1973; Eskew et al., 1977). Tests have shown that over half the strains of some N_2 fixing bacteria are able to denitrify, possibly at the same time as they fix N_2 (Neyra et al., 1977; Zablotowicz et al., 1978).

Denitrifying bacteria occur in over 20 genera (a list is given by Payne, 1981). Most are heterotrophs but some chemolithotrophs are known, e.g. Thiobacillus denitrificans which uses sulphur compounds (Beijerinck, 1904; Baalsrud and Baalsrud, 1954; Baldensperger and Garcia, 1975) and some which use hydrogen (H_2) (Niklewski, 1914; Pfitzer and Schlegel, 1973). Almost all are facultative anaerobes, the only known exception being Propionibacterium pentosaceum which normally grows by fermentation (van Gent et al., 1975).

Although in denitrification, NO_3^- is reduced sequentially to N_2 (see Section 2.3.2), not all denitrifiers are able to perform every step. Some reduce NO_2^- slowly, if at all (Bollag et al., 1970), others can reduce NO_2^- but not NO_3^- (Youatt, 1954; Chatelain, 1969; Pichinoty et al., 1976; Garcia et al., 1977b), and some cannot reduce N_2O (Renner and Becker, 1970; Garcia et al., 1977b; Greenberg and Becker, 1977).

There is no evidence that the organisms most frequently studied in microbiological work (Pseudomonas stuzeri, Pseudomonas perfectomarinus, Paracoccus denitrificans and Pseudomonas denitrificans) are the most numerous or representative. The most numerous denitrifiers from soil were reported to be Bacillus polymyxa, Bacillus sp. Arthrobacter simplex and Micrococcus sp. (Jordan et al., 1967) and Pseudomonas, Bacillus and Micrococcus (Focht and Joseph, 1973). In their definitive study using a wide range of soils from all over the world, Gamble et al. (1977) found that 35% of the isolates were Pseudomonas fluorescens biotype II, with Alcaligenes, Pseudomonas and Flavobacterium (a genus not previously known to contain denitrifiers) being commonly found.

1.2.2. Pathways of Denitrification

Although it was known long ago that during denitrification NO_3^- was reduced to NO_2^-, NO, N_2O and N_2 (Warrington, 1897; Beijerinck and Minkman, 1910; Suzuki, 1912), it took over 70 years for the reduction sequence to be proved beyond doubt.

The existence of an intermediate between NO_2^- and NO was postulated by Cranston and Lloyd (1930) to explain a lag period between NO_3^- and NO_2^- disappearance and the appearance of a gas. They suggested hyponitrite ($N_2O_2^{2-}$) and others considered nitramide ($NO_2.NH_2$) as the intermediate. In bacterial culture Allan and van Niel (1952) found that both $N_2O_2^{2-}$ and $NO_2.NH_2$ were converted to N_2, while Kluyver and Verhoeven (1954) reported little reaction with $N_2O_2^{2-}$ and immediate autodecomposition with $NO_2.NH_2$. Hydroxylamine was also thought by some workers to be an intermediate since it can be reduced by denitrifying bacteria (Asano, 1959) and can be combined with NO_2^- to give oxides of N (Iwasaki and Mori, 1958; Renner and Becker, 1970) by an enzymic process (Iwasaki et al., 1963). However, none of the three compounds has ever been proved to be an intermediate.

Early workers often did not detect N_2O in their experiments (Chung and Najjar, 1956a), probably because their manometric techniques could not detect the small quantities involved and at this time N_2O was not considered an obligatory intermediate. Those who

did detect N_2O (Sacks and Barker, 1952; Verhoeven, 1952; Kluyver and Verhoeven, 1954) considered it to be an alternative end product to N_2 rather than an obligatory intermediate. This view was supported by evidence that although cyanide and azide inhibit N_2O reduction, in their presence NO_2^- could be reduced to N_2 (Sacks and Barker, 1952; Allan and van Niel, 1952; Delwiche, 1959). At a symposium in 1953 Kluyver pointed out that the inhibitors were inactivated by NO_2^- but his results escaped the notice of most workers (Payne, 1981).

There was also doubt as to whether NO is an intermediate since it is unstable and toxic. Although some workers found NO in their incubation experiments with cell cultures (Baalsrud and Baalsrud, 1954; Chung and Najjar, 1956a; Walker and Nicholas, 1961; Cooper and Smith, 1963), it was seldom found in soil incubations except in unusual circumstances, e.g. at low pHs (Cady and Bartholomew, 1960).

The role of N_2O and NO in denitrification was only resolved after the development of gas chromatographic techniques using columns containing 'Porapak' (polystyrene beads) in 1966. This enabled mixtures of NO, N_2O and N_2 to be separated and the improvement of techniques for ^{15}N analysis meant that denitrifying systems could be continuously monitored.

Cady and Bartholomew (1960), who were among the first to use ^{15}N, reported evidence of a sequence involving NO, N_2O and N_2. Matsubara and Mori (1968) showed that N_2O appeared transiently in the reduction of NO_2^- to N_2. Barbaree and Payne (1967) showed by chromatographic analysis that cell-free extracts of Pseudomonas perfectomarinus released NO and N_2O, contrary to previous evidence. Miyat and Mori (1968) detected NO during denitrification in the presence of an inhibitor of NO reduction. Greenberg and Becker (1977), using mutants of Pseudomonas fluorescens lacking N_2O reductase, found that N_2O was the only end product of NO_3^- reduction. In the presence of inhibitors of N_2O reduction the final product of denitrification was N_2O, whether the initial source of N was NO (Garcia, 1977a) or NO_3^- (Matsubari and Mori, 1968; Balderston et al., 1976; Yoshinari and Knowles, 1976).

It is now generally accepted that the sequence of denitrification is:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \quad \text{(Payne, 1981)} \qquad 1.1$$

1.2.3. The Biochemistry of Denitrification

In aerobic respiration, reduced compounds are oxidised by giving up electrons to a chain of electron acceptors, so that as electrons pass down the chain the energy released is used in A.T.P. (adenosine tri-phosphate) formation. The electron chain generally consists of a quinone derivative and cytochromes (proteins containing heme prosthetic groups in which a ferric ion can be reduced to ferrous) and with O_2 as the final electron acceptor. In denitrification NO_3^-, NO_2^-, NO or N_2O replace O_2 in this process.

Aerobic respiration is the most efficient in energy terms: 50-60% of glucose carbon is assimilated, compared to 25% in denitrification and 5-10% in anaerobic respiration (Verhoeven and Goos, 1954).

Growth with an N source in the absence of O_2 proves the link between denitrification and A.T.P. synthesis. Denitrifying organisms have been grown on NO_3^- and NO_2^- (Bollag et al., 1970; Focht and Joseph, 1973; Doner et al., 1975; Gamble et al., 1977 ; Garcia, 1977a) and on N_2O (Matsubara, 1975; Pichinoty et al., 1976; Garcia et al., 1977b; Sørenson et al., 1980) but so far only Pichinoty et al. (1978, 1979) have reported growth on NO due to its toxicity. Koike and Hattori (1975a) showed that the growth yield per mole of NO_3^- was approximately half that of O_2. Since 1 mole O_2 and NO_3^- accepts 4 and 5 moles electrons respectively, they inferred that there were fewer phosphorylation sites when NO_3^- was the final electron acceptor.

$$O_2 + 4e^- + 4H^+ = 2H_2O \qquad 1.2$$

$$NO_3^- + 5e^- + 6H^+ = \tfrac{1}{2}N_2 + 3H_2O \qquad 1.3$$

They also showed that NO_3^-, NO_2^- and N_2O gave growth yields of 28.6, 16.9 and 8.8 g $mole^{-1}$ respectively, the results for NO_3^- and NO_2^- roughly corresponding to the oxidation number of N (5:3). They concluded that there are a similar number of phosphorylation sites

for NO_3^- and NO_2^- and rather more for N_2O (oxidation number 1) (Koike and Hattori, 1975b). Since the N_2O:N_2 couple has a greater potential than the other steps, this is not surprising.

Eo = 1.35V	N_2O/N_2
= 0.42V	NO_3^-/NO_2^-
= 0.77V	NO_2^-/N_2O

E_o is the potential of the couple at 25°c and 1atm (Kristjansson and Hollocher, 1980)

Cell free extracts of the enzymes responsible for denitrification have been isolated from whole cells, i.e. NO_3^- reductase (Forget and DerVartanian, 1972; Baldensperger and Garcia, 1975; Calder et al., 1980) NO_2^- reductase (Radcliffe and Nicholas, 1968; Payne et al., 1971) NO reductase (Cox et al., 1971; Cox and Payne, 1973; Zumft, 1979) and N_2O reductase (Matsubara and Mori, 1968; Barbaree and Payne, 1967; Payne and Riley, 1971). Using the extracts the sequence of denitrification (Equation 1.1) has been confirmed. Work with cell free extracts of NO_2^- reductase has shown that NO is the product of reduction even though NO is not usually released from whole cells; i.e. NO normally remains bound within the cell and is therefore seldom released in soils (Payne, 1981).

1.2.4. The Effects of Acetylene on Microbial Processes

1.2.4.1. Denitrification

The initial discovery that C_2H_2 inhibits N_2O reduction (Federova et al., 1973) has been confirmed in bacterial cultures (Balderston et al., 1976; Yoshinari and Knowles, 1976) and in soils by the use of ^{15}N (Paul and Victoria, 1978) and ^{13}N (Smith et al., 1978).

Sulphide, also a known inhibitor of N_2O reduction (Sørenson, 1978; Myers, 1972) removes the effect of C_2H_2 (Tam and Knowles, 1979) as does sulphur-containing protein, and organic substances high in sulphur (Yeomans and Beauchamp, 1982b). Thus in any sulphide containing environment, C_2H_2 may be ineffective as an inhibitor.

The conversion of NO_3^- to N_2O in the presence of C_2H_2 is stoichiometric in bacterial cultures (Yoshinari and Knowles, 1976) and soils (Yoshinari et al., 1977). The rate of NO_3^- reduction is unaffected by C_2H_2, although NO_2^- reduction is slightly slower

(Yoshinari and Knowles, 1976), in spite of the fact that Cho (1982) predicted a 25% increase in NO_3^- reduction from theoretical considerations.

Where C_2H_2 concentrations below $1x10^{-2}$ ml ml^{-1} were used inhibition was reversible in bacterial cultures (Balderston et al., 1976) and in soils (Ryden et al., 1979a; Yeomans and Beauchamp, 1978). However at concentrations greater than $1x10^{-2}$ ml ml^{-1} the effect was irreversible (Balderston et al., 1976).

Reported C_2H_2 concentrations required for complete inhibition in soils range from $1x10^{-3}$ to $2x10^{-2}$ ml ml^{-1} (Yoshinari et al., 1977; Sørenson, 1978; Germon, 1980a; Ryden, 1982a). However, in bacterial culture different organisms required different concentrations for complete inhibition (Yoshinari and Knowles, 1976). The concentration required appears to depend on the duration of the experiment, higher concentrations being required to prolong the inhibitory effect (Yeoman and Beauchamp, 1978). Germon (1980a) found that even at $2x10^{-2}$ ml ml^{-1} C_2H_2, inhibition was incomplete after two weeks. Higher NO_3^- concentrations, which result in more N_2O concentrations increase the C_2H_2 concentration required and in some circumstances inhibition is not complete even under a pure C_2H_2 atmosphere (Smith et al., 1978).

From the above, it appears that concentrations of $1x10^{-3}$ ml ml^{-1} C_2H_2 may be adequate for complete inhibition at low NO_3^- concentrations over a short period of time but $1x10^{-2}$ ml ml^{-1} would be required at higher NO_3^- concentrations. Continuous use of C_2H_2 would not be totally effective.

1.2.4.2. Respiration

Although few studies have specifically looked at the effects of C_2H_2 on respiration, some workers have measured carbon dioxide (CO_2) evolution when using C_2H_2 in experiments on denitrification. In some soil incubation experiments the presence of C_2H_2 had no effect on CO_2 production (Ryden et al., 1979a; Smith et al., 1978; Ryden, 1982). In contrast Klemedtsson et al. (1977) found a 13% increase in CO_2 production in the presence of C_2H_2, which was statistically

significant, but offered no explanation. His incubations were carried on for three weeks after the disappearance of NO_3^- - much longer than in other work.

Enhanced respiration may be due to the presence of microorganisms able to use C_2H_2 as a carbon source, either immediately or after adaptation. Organisms are known which are able to use lower alkenes, including ethylene (C_2H_4) (Zobell, 1950; de Bont, 1975). Birch-Hirschfeld (1932) reported identifying an organism as Mycobacterium laticola, able to use C_2H_2 but not C_2H_4, methane (CH_4) or propylene (C_3H_6) as an energy source, but McKenna and Kallis (1965) state in their review that this was not confirmed by other workers.

De Bont (1976) and Germon (1980b) noted C_2H_2 disappearance during prolonged soil incubations and suspected its use by microorganisms. Watanabe and de Guzman (1980) showed that this occurred in both aerobic and anaerobic environments but only in unsterilised soil. During C_2H_2 consumption no C_2H_4 formed, but increased concentrations of fatty acids indicated a fermentation process. Other workers also found increased CO_2 production, but no C_2H_4, in the presence of C_2H_2 and suggested that C_2H_2 was only consumed in the absence of any other carbon source (Germon, 1980b; Yeomans and Beauchamp, 1982a). Culbertson et al. (1981) were the first to use labelled ^{14}C and showed that $^{14}C_2H_2$ was converted to $^{14}CO_2$ by estuarine sediments in anaerobic conditions.

So far in pure culture only aerobic C_2H_2 utilizing bacteria have been isolated and the evidence is that the ability is limited to a few organisms. Kanner and Bartha (1979) have isolated and identified Nocardia rhodocrous which could use a variety of conventional carbon sources as well as C_2H_2, but not C_2H_4, and had a growth requirement for a thiamine vitamin. De Bont and Peck (1980) isolated and identified an organism Rhodococcus Al, which could use C_2H_2 or many conventional substrates but not lower alkanes or C_2H_4, and which had no vitamin requirement. Both authors claim that their organism was the same as that of Birch-Hirschfeld (1932). Both de Bont and Peck (1980) and Kanner and Bartha (1982) found acetaldehyde ($CH_3.CHO$) as an intermediate in C_2H_2 consumption, indicating that C_2H_2 is metabolised through a hydration reaction. This seems

the most likely pathway since no evidence has been reported for a nitrogenase-type reduction to C_2H_4 and no C_2H_2 utilizers have been able to use C_2H_4 as an alternative substrate.

1.2.4.3. Nitrogen Fixation and Methane Production and Utilisation

Acetylene has been used since the 1960's in assays of N fixation because it acts as a competitive substrate, being reduced to C_2H_4 which is easily measured by gas chromatography (Dilworth, 1966). Therefore, when C_2H_2 is used in the field, N fixation will be inhibited, and soil NO_3^- concentrations may therefore be affected.

Raimboult (1975) showed that C_2H_2 prevented the growth of Methanosarcina, a bacterium responsible for CH_4 production in sediments. Acetylene also inhibits the oxidation of CH_4 and other simple alkanes, and the co-oxidation of C_2H_4 by interfering with CH_4 hydroxylase by competitive inhibition (De Bont and Mulder, 1976).

1.2.4.4. Nitrification

Acetylene has been shown to inhibit nitrification totally at concentrations as low as $5x10^{-3}$ ml ml^{-1} (Ryden, 1982)and $1x10^{-3}$ ml ml^{-1} (Walter et al., 1979) in soils and $1x10^{-5}$ ml ml^{-1} in culture studies (Hynes and Knowles, 1978). The inhibition is reversible after a lag period (Walter et al., 1979). Although in theory this might lead to low NO_3^- concentrations when C_2H_2 is used in field experiments, such an effect has not been found in practice (Ryden and Dawson, 1982).

1.3. Soil Aeration

Gas phase concentrations in this section are given as ml ml^{-1} and concentrations in the aqueous phase as the concentration in the gas phase which would be in equilibrium with the solution.

1.3.1. The Mechanism of Oxygen Supply to Soils

At some critical O_2 concentration aerobic respiration ceases and anaerobic processes begin. Using a stirred soil suspension to avoid O_2 concentration gradients, Greenwood (1963) found that the critical O_2 concentration was $3.2x10^{-3}$ ml ml^{-1} ($4x10^{-6}$M). He also found the

critical O_2 concentration within saturated aggregates of uniform size by calculating from diffusion theory the proportion of the volume of the aggregates which were aerobic, and hence calculating a theoretical respiration rate. By measuring actual respiration he found that the critical O_2 concentration was 6×10^{-4} ml ml^{-1} (0.7×10^{-6}M) (Greenwood, 1961).

Several workers (Currie, 1961; Leffelaar, 1979; Smith, 1977, 1980) have visualised the soil as a collection of aggregates of varying sizes, with the inter-aggregate pores forming a system of macropores which are usually air filled. Within the aggregates, where most nutrient uptake, root growth, and microbial activity, and therefore respiration, takes place, there is a system of micropores, the proportion of air filled and water filled pores depending on the soil moisture tension. Oxygen is therefore supplied to the site of respiration from the atmosphere through the inter-aggregate and intra-aggregate pores.

Mass flow (the response to a pressure gradient) and diffusion (the response of a component of a gaseous mixture to a partial pressure, i.e. concentration, gradient) are the two main mechanisms of gaseous exchange.

Mass flow due to temperature lags and barometric changes between the surface and subsoil has been estimated to account for less than 0.5 and 1% of the required O_2 respectively (Rommel, 1922; Buckingham, 1904). The action of wind was once thought to have little contribution (Buckingham, 1904) though more recent work (Kimball and Lemon, 1971; Scotter et al., 1967; Farrell et al., 1966) shows that wind turbulence may cause some mixing of soil air in the top few centimetres of a bare unconsolidated soil.

In the soil atmosphere, the O_2 deficit (the difference between the measured O_2 concentration and that of ambient air) is greater than the CO_2 concentration, due to the greater solubility of CO_2, which gives rise to more rapid diffusion of CO_2 away from a site of respiration than diffusion of O_2 to the site, and loss of CO_2 in percolating water (Boyce and McCalla, 1969). This leads to an overall pressure reduction and to mass flow (Wood and Greenwood, 1971) but although the contribution of this mechanism to O_2 exchange has not been estimated, it is likely to be small.

Diffusion is therefore the major mechanism for gaseous exchange in soil. As O_2 is consumed partial pressure gradients cause O_2 to diffuse towards the area of consumption, according to Fick's 1st Law (Fick, 1855):

$$F = -D\partial c/\partial x \qquad 1.4$$

where F is the flux of gas

$\partial c/\partial x$ is the concentration gradient in the direction x

D is the diffusion coefficient (for O_2 in air $D = 0.2 cm^2 s^{-1}$, and in water = $0.2 \times 10^{-4} cm^2 s^{-1}$)

Equation 1.4 applies in both liquid and gaseous media but diffusion in water is approximately 10,000 times slower than in air. Since the solubility of O_2 is low, concentration gradients in water are also lower than in air and therefore diffusion through water filled pores often does not match the O_2 demand. Therefore the adequacy of the O_2 supply to the soil depends largely on the air filled porosity which in turn depends on the moisture tension.

Fick's 2nd Law (Fick, 1855) shows how concentration varies with time (i.e. where the conditions are not steady state). Where there are sources or sinks of the diffusing gas the law is amended to:

$$\partial c/\partial t = D\,\partial^2 c/\partial x^2 + Q \qquad 1.5$$

where Q is the rate of production of the gas (i.e. negative for O_2)

Standard solutions of Equation 1.5 have been worked out for special conditions but they represent an oversimplification of the real conditions in field soils (van Bavel, 1951, 1952; Wesseling, 1962; Papendick and Runkles, 1965; Crank, 1975).

Inter-aggregate air filled macropores are more likely to contain adequate O_2 concentrations while micropores, particularly in the centre of large aggregates are more likely to be anaerobic. It has been known for some time that anaerobic microsites can exist in soils with well aerated macropores (Russell and Appleyard, 1915; Jansson and Clarke, 1952; Skerman and Macrae, 1957).

Although the diffusion coefficient, as measured in a soil, is that for the inter-aggregate pores, it is a crucial factor in determining whether aeration is adequate. There have been many attempts to relate the diffusion coefficient (D) to the diffusion coefficient in air (D_o) and air filled porosity (S), a fairly easily measured variable. If diffusion were simply restricted by the available soil air space, D would be given by:

$$D = SD_o \qquad 1.6$$

In practice, D is less than expected because of tortuosity in pores and because of blocked pores etc. Various relationships have been suggested:

$D = D_o S^2$ (Buckingham, 1904) 1.7

$D = 0.66\ SD_o;\ 0 < S < 0.7$ (Penman, 1940) 1.8

$D = (0.9S - 0.12)D_o$ (Wesseling and van Wijk, 1957) 1.9

$D = S^{4/3}D_o$ (Millington, 1959) 1.10

$D = \gamma S^{\mu}D_o$ (Currie, 1960) 1.11

where $\gamma = 0.8\text{-}1.0$, increasing with mean porosity.

μ is a measure of pore shape.

Penman's formula (1.8) has been widely used but it is not valid when S is low, since many pores are then blocked and D becomes very low (Blake and Page, 1948; Wesseling and van Wijk, 1957; Grable and Siemer, 1968). Wesseling's equation (1.9) assumes that 12% of the pore space is blocked. No one equation seems valid for all soils.

Various methods have been used for measuring diffusion coefficients. Blake and Page (1948) and Domby and Kohnke (1956) calculated the diffusion coefficients from the weight loss of carbon disulphide as it evaporated and diffused through soil in a chamber. In Taylor's method (1949) O_2 diffused through the soil into a N_2 filled chamber, and D was calculated from the increase in O_2 concentration. Field methods were developed to measure D from the rate of diffusion of O_2 into a buried chamber of N_2 (Raney, 1950) and away from an access needle in the ground (Lai et al., 1976). More recently methods

involving the diffusion of radioactive krypton -85 through soil cores have been developed (Wellar et al., 1974; Ball, 1979).

Diffusion equations have been solved for intra-aggregate diffusion by Currie (1961) and by Greenwood (1961). For a spherical aggregate or crumb, Greenwood and Berry (1962) give the relationship:

$$6D(C_1-C_o) = M(R_1^2+2R_o^3/R_1-3R_o^2) \qquad 1.12$$

where C_1 is the concentration of O_2 at the crumb surface.

C_o is the critical O_2 concentration below which anaerobic processes occur.

M is the rate of respiration.

R_1 is the crumb radius.

R_o is the radius at which O_2 becomes critical.

If C_o is taken as 0, and R_o = 0, i.e. the crumb is totally aerobic; equation 1.12 can be reorganised to give the maximum radius for a completely aerobic crumb:

$$R_1 = \sqrt{(6DC_1/M)} \qquad 1.13$$

Greenwood and Goodman (1967) found reasonably good agreement with the theory using moulded soil spheres, when the gas filled pore space in the spheres was low.

Greenwood (1963) predicted that in an arable soil where aggregate sizes are less than 1cm radius with an air filled porosity greater than 10%, anaerobic microsites would be unlikely to occur. Anaerobic microsites are more likely when poor structure increases aggregate sizes, when the air filled porosity is less than 10%, or when respiration rates are very high, e.g. when dry soil is remoistened or organic materials are added to the soil.

Currie (1961), and Smith (1977), using data from Currie (1965), found values of D an order of magnitude less than Greenwood and Goodman (1967), corresponding to a 3-fold decrease in the critical radius for a completely aerobic crumb.

Smith (1977) has used Currie's and Greenwood's work to model the extent of anaerobiosis, given that aggregate sizes are log normally distributed. His model shows that D is more important than aggregate

size in determining the proportion of the soil volume which is anaerobic.

1.3.2. Soil Aeration Measurements

No quantitative measurement is entirely satisfactory in the assessment of soil aeration status because of the complex nature of soil structure.

One measurement used is the oxygen diffusion rate (o.d.r.) (Lemon and Erickson, 1962; Clark et al., 1953; Willey and Tanner, 1963; Willey, 1974), which measures the flux of O_2 to a cathode, where O_2 is reduced to hydroxyl ions. The current depends on pH, salt content and ion species as well as O_2 diffusion (McIntyre, 1970) and there is difficulty in obtaining readings in dry soil (Flühler et al., 1976a). However, the o.d.r. does measure the ability of the soil to supply O_2 to a site of respiration. Quoted critical rates for the maintenance of adequate aeration range from 15×10^{-8} to 40×10^{-8} g cm^{-2} min^{-1} (Betrand and Kohnke, 1957; Letey et al., 1962; Stolzy and Letey, 1964; Dilkova and Galeva, 1978).

The redox potential (r.p) (the potential difference between a standard hydrogen electrode and an inert electrode placed in the soil) has also been used as an index of soil aeration. When soil becomes anaerobic the r.p. falls in stages, remaining constant for a while after each fall, when it is said to be 'poised' and one ion species in the solution is being reduced. Ions responsible for poising include NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-} in order of decreasing r.p. The quoted r.p. for NO_3^- ranges from 200 mV to 650 mV (Patrick, 1960; Bell, 1969; Pilot and Patrick, 1972; Armstrong, 1975; van Cleemput et al., 1975; Reddy and Patrick, 1975; Ryden and Lund, 1980a; Letey et al., 1981). This wide range, and the high variability of r.p. over very small distances (Flühler et al., 1976b) means that the r.p. is difficult to interpret and of limited value in the absence of other information.

Soil atmospheric analysis for O_2 and CO_2 is the most commonly used method of determining aeration status. Sampling devices of

small volume, e.g. a long needle (van Bavel, 1965) or a capillary tube (Tackett, 1968), which rely on drawing air from the soil macropores, are easily blocked and difficult to use in wet soil. Usually a reservoir containing air is buried in the soil, into which gases diffuse and from which samples can be withdrawn to the soil surface. In early work reservoirs were large, and samples were withdrawn using a pump, since before the advent of chromatography large samples were required for analysis (Russell and Appleyard, 1915; Boynton and Reuther, 1938; Furr and Aldrich, 1943; Robinson, 1957; Epstein et al., 1957; van Bavel, 1965). The use of a pump causes degassing of CO_2 from the soil solution and therefore enhanced CO_2 concentrations (Taylor and Abrahams, 1953). Later, reservoirs were smaller, consisting for example, of glass tubing (Yamaguchi et al., 1962), a 30ml can connected to capillary tubing (Burford and Millington, 1968), and a pipe coupling connected to galvanised pipe (Tackett, 1968), all of which were sampled by means of syringes. Dowdell et al. (1972) used reservoirs consisting of sintered bronze cups (5μm pores), porous to air, and also water at low soil moisture tensions.

Reservoirs should be large enough so that sampling does not cause appreciable pressure reduction, but small enough so that equilibrium between soil gases and the reservoir atmosphere is rapidly established after sampling, and of volume much larger than the connecting tubes to the surface. Gas concentrations in such reservoirs are in equilibrium with the soil macropore atmosphere and can only give an indication of concentration of gases within aggregates, where respiration takes place.

1.3.3. Oxygen and Carbon Dioxide Concentrations in Soil

Oxygen concentrations can vary from 0 to 0.21 ml ml^{-1} and CO_2 from 0 to over 0.12ml ml^{-1} (Boynton and Reuther, 1938; Furr and Aldrich, 1943; King, 1982). Mass flow, caused by the O_2 deficit being greater than the CO_2 concentration (see Section 1.3.2.) leads to an increased N_2 content in the soil atmosphere over atmospheric concentrations (Yamaguchi et al., 1962; Boyce and McCalla, 1969; Wood and Greenwood, 1971) and concentrations of N_2 up to 0.90 ml ml^{-1} have been

measured (Furr and Aldrich, 1943; Taylor and Abrahams, 1953).

Although respiration is higher in the summer, in most temperate regions poor aeration occurs in winter (Furr and Aldrich, 1943; Dowdell et al., 1979a) or spring (Russell and Appleyard, 1915), since the soil is then wetter. They found that in the winter O_2 concentrations varied with temperature while summer concentrations were affected more by rainfall. In his review Smith (1977) states that heavy clay soils are usually anaerobic in the winter or spring but peaty-gley soils may be anaerobic in the summer.

The variation of O_2 and CO_2 concentrations with depth depends on changes in O_2 demand and soil texture with depth, and the height of the water table. In typical clay soils in Britain where the water table is high in winter, O_2 concentrations decrease and CO_2 increases with depth (Boynton and Reuther, 1938; Taylor and Abrahams, 1953; Dowdell et al., 1979a). In very deep light textured soils, such as those of the central Valley of California, there is often a deep reservoir of aerated soil, and anaerobic conditions are very much a surface phenomenon caused by temporary waterlogging following rain or irrigation (Furr and Aldrich, 1943; Rolston et al., 1976a).

The addition of crop residues or farmyard manure to soil has sometimes been found to decrease O_2 and increase CO_2 concentrations in the soil profile, by providing mineralisable organic matter and therefore increasing O_2 demand (Epstein et al., 1957; Wallingford et al., 1975; Focht et al., 1979). The addition of liquid manure (slurry) may have a much greater effect, since large volumes of water are applied as well as available organic matter. Burford (1976), who applied a large amount of slurry (550 t ha^{-1}) in the spring found that for about two weeks gaseous exchange was prevented by the 4cm layer of wet slurry overlying the soil surface, leading to a decrease in O_2 concentration. Thereafter, O_2 concentrations at the 10cm depth remained below 0.10 ml ml^{-1} for 4 months, and then increased to 0.19 ml ml^{-1} by October. Reduced concentrations were due to the mulching effect of the slurry layer, reducing water evaporation and increasing the moisture content throughout the soil profile (Thijell and Burford, 1975). Following the ploughing in of the slurry layer in October,

during the winter O_2 concentrations were about 0.02-0.03 ml ml^{-1} lower in the slurried than in the control plot below the 10cm depth. Concentrations of CO_2 were also increased by the slurry, reaching 0.17 ml ml^{-1} at 10cm depth. Burford's work illustrated the effect of dumping slurry rather than using it as a fertiliser, for which application rates of up to 40 t ha^{-1} are recommended (Tunney, 1981). Such quantities may have a temporary effect on aeration, similar to a heavy fall of rain, but would be unlikely to have severe long term effects.

1.4. Laboratory Studies of Denitrification in Soil

1.4.1. Methods

Until recently, denitrification has largely been studied by incubating soil under controlled conditions. This has given much valuable, although not always unequivocal, information on the denitrification process, and the potential of various soils for denitrification. This work is reviewed in Sections 1.4.2. and 1.4.3.

The use of columns packed with soil allows the investigation of denitrification in conditions more like those in the field, while allowing soil moisture content, soil N, and temperature to be closely controlled. Denitrification has been investigated using columns by

measuring the NO_3^- concentration profile (Mann et al., 1972; Ardakani et al., 1975; Doner et al., 1975; Volz and Starr, 1977; Hynes and Knowles, 1980; Christenson, 1980), the N_2O and ^{15}N labelled N_2 concentration profiles (Rolston et al., 1976) or N_2O and N_2 fluxes (Gilliam et al., 1978; Overrein, 1968). Columns of soil have been used to investigate the fate of N in sewage effluent applied to the soil (Jacobson and Alexander, 1980; Gilbert et al., 1979), and the population dynamics of denitrifying bacteria (Ardakani et al., 1975; Doner et al., 1975; Starr and Parlange, 1975; Volz et al., 1975; Volz and Starr, 1977; Christenson, 1980; Jacobson and Alexander, 1980), and to test models of NO_3^- and NH_4^+ transport in soils (Misra et al., 1974a; Starr et al., 1974; Rolston and Marino, 1976; Reddy et al., 1976).

1.4.2. The Potential of Soils for Denitrification

No surface soil has yet been reported as totally unable to denitrify when incubated, although various factors, e.g. pH (Balasubramanian and Kanehiro, 1976), lack of organic substrate (Macgregor, 1972; Greenland, 1962) or lack of NO_3^- (Macgregor, 1972) may severely limit denitrification. Denitrifying bacteria were found in all of 19 soils and 3 lake sediments from 8 countries, and including mineral, organic and waste treated soils, from rice paddies, temperate agricultural areas, and rainforests (Gamble et al., 1977). Even in soils where conditions for denitrification are found rarely, if ever, e.g. desert soils (Macgregor, 1972) denitrification occurs in anaerobic conditions. The potential for denitrification has been found in such diverse environments as tundra (Barsdate and Alexander, 1975), Hawaiian tropical soils (Balasubramanian and Kanehiro, 1976), spodosols and peats in South Finland (Müller et al., 1980), solodised solonetz soils in Eastern Australia (McGarity and Myers, 1968) and Ghanaian cultivated forest and old grassland soils (Greenland, 1962).

1.4.3. The Kinetics of Denitrification

Most research has centred on the rate of NO_3^- reduction rather than on other stages in the denitrification process.

Since denitrification is an enzymatic process, Bowman and Focht (1974) suggested that NO_3^- reduction would follow dual substrate Michaelis-Menten kinetics:

$$V = V_{max} (C/(C+K_c)) ([NO_3^-\text{-}N]/([NO_3^-\text{-}N] + K_N)) \qquad 1.14$$

where V is the rate of NO_3^- reduction

V_{max} is the maximum rate when neither N nor C is limiting

C is the concentration of the carbon source

K_c is the Michaelis constant for the carbon source

$[NO_3^-\text{-}N]$ is the nitrate concentration

K_N is the Michaelis constant for NO_3^-

From theory, the Michaelis constant is the reciprocal of the equilibrium constant for the formation of the enzyme complex and should therefore be fairly similar in all soils. Although some workers have reported a good fit to equation 1.14, reported values of K_N vary widely, as shown by Table 1.1, probably because one condition for the equation to hold is that no new enzyme is synthesised and the microbial population remains constant during the experiment.

Table 1.1 Reported values of the Michaelis constant for nitrate reduction

Source	Reported value of K_N ($\mu g\ g^{-1}$)	Energy Source
Yoshinari et al., 1977	6	with glucose
	0.7	no glucose
Klemedtsson et al., 1977	4	no C source
Bowman and Focht, 1974	170	2 soils with glucose
	1,700	

Michaelis-Menten kinetics simplify to first order kinetics when $[NO_3^-\text{-}N]$ is much less than K_N, i.e. the rate of reduction is proportional to the NO_3^- concentration:

$$V = K\,[NO_3^- - N] \qquad 1.15$$

where V, $[NO_3^- - N]$ are as above

K is the first order rate constant

First order kinetics were found to fit NO_3^- reduction for concentrations up to 200 $\mu g\ ml^{-1}$ in 16 different soils in stirred suspensions by Reddy et al. (1982) and up to 100 $\mu g\ g^{-1}$ by Stanford et al. (1975c). Kohl et al. (1976) found limits of 300 $\mu g\ g^{-1}$ in a cultivated soil but only 10 $\mu g\ g^{-1}$ in an uncultivated one. As predicted by diffusion theory, first order kinetics occurs when denitrification is limited by diffusion, e.g. where the soil layer has an overlying layer of water or where NO_3^- must diffuse into the anaerobic centre of an aggregate (Stanford et al., 1975b; Reddy et al., 1978).

Where NO_3^- concentrations are high, equation 1.14 simplifies to zero order kinetics, i.e. the rate is independent of NO_3^- concentration.

$$V = K \qquad 1.16$$

where K is the zero order rate constant

Zero order rates have been reported at concentrations as low as 5 $\mu g\ g^{-1}$ (Patrick, 1960) and 3 $\mu g\ ml^{-1}$ (Reddy et al., 1978).

The range of rates of denitrification in incubation experiments is very high (Tables 1.2-1.4). However, there is no standard incubation procedure and different methods have been used to prepare soil, achieve anaerobic conditions and measure denitrification.

Rates of up to 5.3 $\mu g\ g^{-1}h^{-1}$ and 16.9 $\mu g\ g^{-1}h^{-1}$ have been reported in the absence and presence of added energy sources respectively. Müller et al. (1980) found 400-fold differences in the rate of denitrification in 22 soils. Tables 1.2-1.4 show a low potential for denitrification in soils of inherently low fertility (e.g. Müller et al., 1980) and where the conditions for denitrification occur rarely (e.g. MacGregor, 1972).

Few studies to investigate rates of N_2O reduction have been carried out (Table 1.2) but rates appear to be similar to those for NO_3^- reduction. Letey et al. (1981), however, report higher rates

Table 1.2. Measured rates of NO_3^- reduction by topsoil with no added energy source

Source	Measured Rate ($\mu g\ N\ g^{-1} h^{-1}$)	NO_3^- added ($\mu g\ N\ g^{-1}$)	Soils	Conditions of Incubation	Parameter Measured
Smith et al., 1978	0.8	8	Brookston loam pH 7.6	Soil slurry in He or Ar atmosphere at 22°C in the dark	N_2O in presence of C_2H_2 after 3-10 hrs
	2.2	0	Carlisle muck pH 6.5		
	0.4	0	Spiriks loamy sand pH 6.4		
			Stored field moist at 2°C		
Knowles, 1979	0.41-0.62	28	Sediment from Lake St.George Ontario, stored at 4°C	In He atmosphere	N_2O in presence of C_2H_2
Müller et al., 1980	0.005-2.24 ($\mu g\ N\ ml^{-1}\ h^{-1}$)	42	22 low pH spodosols and peats of Finland, 19 non-agricultural, 3 agricultural stored at -18°C	Waterlogged soil in N_2 atmosphere after pre-incubation to remove indigenous NO_3^-	N_2O in presence of C_2H_2
Yoshinari et al., 1977	0.9	70	Sandy loam from Quebec	Moist soil in He atmosphere at 24°C in the dark	N_2O in presence of C_2H_2

/...

Table 1.2 contd. - 2

Source	Measured Rate ($\mu g\ N\ g^{-1}h^{-1}$)	NO_3^- added ($\mu g\ N\ g^{-1}$)	Soils	Conditions of Incubation	Parameter Measured
Stanford et al., 1975b	0.078-1.24	80	30 diverse soils of differing pH, organic matter etc. stored air dry	With overlying water in stoppered tubes initially aerobic, at 35°C	NO_3^- disappearance (average rate over period is quoted)
McGarity and Myers, 1968	0.12-5.73	100	17 solodised solonetz soils of E.Australia Stored at 2.5°C	Saturated aggregates in Ar atmosphere at 30°C	N_2 and N_2O during steady phase of denitrification
Greenland, 1962	0.42-1.88	125-200	Ghanaian cultivated and uncultivated soils	Soil with overlying water	NO_3^- disappearance
MacGregor, 1972	0.002	0	Uncultivated Sonoran desert soil (sandy loam)	At 60% of water holding capacity in Ar atmosphere at 38°C	N_2 and N_2O
	0.08	270			
	0.21	0	Cropped clay loam stored air dry		
	0.81	270			

/...

Table 1.2 contd. - 3

Source	Measured Rate ($\mu g\ N\ g^{-1}h^{-1}$)	NO_3^- added ($\mu g\ N\ g^{-1}$)	Soils	Conditions of Incubation	Parameter Measured
Balasubramanian and Kanehiro, 1976	0.07-0.47	390	6 soils of range pH and organic matter of Hawaii	Saturated soil in He atmosphere	N_2 and N_2O
Dubey and Fox, 1974			Semi-tropical soils of Puerto Rico air dried and sieved:	Waterlogged soil in He atmosphere at 23°C	N_2 and N_2O after 1 week
	0.28	400	pH 5.2 sandy clay loam		
	0.18	400	pH 4.5 sandy clay loam		
	0.49	400	pH 5.9 clay		

Table 1.3. Measured rates for the reduction of nitrate by topsoil with added energy sources

Source	Measured rate $\mu g\ N\ g^{-1}h^{-1}$	Soils	Nitrate added $\mu g\ N\ g^{-1}$ soil	Conditions of Incubation	Parameter measured
Yoshinari et al., 1977	2.9	Sandy loam from Quebec	70	Moist soil in He atmosphere at 24°C in dark with 1mg g^{-1} glucose added	N_2O in the presence of C_2H_2
Westerman and Tucker, 1978	0.7	Sonoran desert soil (Sonoita sandy loam) crushed and sieved	100(^{15}N)	Saturated soil at 37°C with 15mg g^{-1} glucose added	$^{15}NO_3^-$ disappearance allowing for ^{15}N-organic fraction
Reddy et al., 1978	4.0-6.1	Range of soils from U.S.A. of pH range 4.7-7.6 and total carbon 0.7-3.1% stored air dried	100	Saturated soil in Ar atmosphere with rice straw	NO_3^- disappearance after 24h (since in some soils all NO_3^- disappeared, rates may be an underestimate)
Greenland, 1962	1.13-4.58	Ghanaian cultivated and uncultivated soils	125-200	Waterlogged soil with overlying water and .15-.2 mg g^{-1} glucose added	NO_3^- disappearance

/...

Table 1.3 contd.

Source	Measured rate $\mu g\ N\ g^{-1} h^{-1}$	Soils	Nitrate added $\mu g\ N\ g^{-1}$ soil	Conditions of Incubation	Parameter measured
MacGregor, 1972	0.26	Sandy loam - uncultivated soil from Sonoran desert	270 (^{15}N)	Soil at 60% water holding capacity in He atmosphere at 38°C with .15 mg g^{-1} glucose added	N_2 and N_2O concentrations
	2.43	Clay loam - cropped soil			
Khan and Moore, 1968	1.75	Alberta soils - air dried and ground: Peat - cultivated	400	Soil at cm tension in He atmosphere with 25 mg g^{-1} glucose added	Difference between total gas evolved with and without NO_3^- + glucose
	7.00	- uncultivated			
	2.88-4.3	6 mineral soils - cultivated			
	4.00-16.9	uncultivated			

Table 1.4. Measured rates of reduction of nitrous oxide

Source	Measured Rate[(a)] ($\mu g\ N\ g^{-1} h^{-1}$)	Soils	Conditions of Incubation
Garcia, 1974	3.5	Rice soil of Senegal	Saturated soil in the absence of O_2. Rate quoted is a maximum rate (Reduction followed Michaelis Menten Kinetics)
Yoshinari et al., 1977	0.78	Sandy loam from Quebec	Moist soil in the atmosphere preincubated to remove NO_3^-
Blackmer and Bremner, 1978	3-7	Range of Iowa soils of various texture, pH and organic matter. Air dried.	Saturated soil in He atmosphere in absence of NO_3^-

(a) All rates were calculated from the rate of disappearance of N_2O.

for the former process.

Even for soils with no added energy source, measured rates of over 1μg N $g^{-1}h^{-1}$ are common - equivalent to a loss of 3.9kg N $ha^{-1}h^{-1}$ or 94kg $ha^{-1}d^{-1}$ by denitrification (if such a rate prevailed in the top 30cm of a soil of bulk density 1.3). The implications for agriculture are obvious.

1.4.4. Factors Affecting Denitrification

1.4.4.1. Moisture Content

The effect of moisture content on aeration and therefore denitrification depends on the O_2 demand and supply. In work by Myers and McGarity (1972) NO_3^- losses increased if an energy source was added to the soils at the same moisture content. In incubation experiments the O_2 supply depends on the O_2 concentration, the volume of the headspace and the geometry of the soil mass, e.g. soil in a petri dish may be saturated but aerobic.

Denitrification has been reported at moisture contents as low as 8-16% (by weight) (Stefanson and Greenland, 1970) and 15-20% of water holding capacity (Arnold, 1954; Ekpete and Cornfield, 1964). Rates of denitrification increase with moisture content (Bremner and Shaw, 1958b; Ekpete and Cornfield, 1964; Stefanson and Greenland, 1970; van Cleemput, 1971; Burford and Stefanson, 1973; Crasswell and Martin, 1974; Dubey and Fox, 1974), although rates may decrease when the moisture content is very high (Arnold, 1954).

Denitrification rates often increase rapidly at some critical moisture content e.g. at field capacity (Westerman and Tucker, 1978) just above field capacity (Mahendrappa and Smith, 1967; Stefanson, 1973) at 70% of water holding capacity (Bremner and Shaw, 1958b), and at around saturation (Meek et al., 1962; Garcia, 1974).

There is some evidence that at moisture contents optimal for denitrification NO_3^- is the preferred substrate, i.e. N_2O builds up, while at higher moisture contents N_2O is reduced as rapidly as NO_3^- (Mahendrappa and Smith, 1967; Cho and Sakdinan, 1978). This may be due to slower diffusion of N_2O into the vessel headspace thereby increasing the chance of N_2O being reduced (Letey et al., 1980a).

Moisture content, air filled porosity and soil moisture tension are all related. Values of air filled porosity below which denitrification takes place have been quoted as 10% (20cm tension) (Letey et al., 1980b) and 11% for a sandy loam and 14% (40cm tension) for a silty clay loam (Pilot and Patrick, 1972).

In incubation under He and Ar, i.e. where moisture content does not affect aeration, Mahandrappa and Smith (1967) found that denitrification rates were unaffected by moisture content, but Cady and Bartholomew (1960) found a threefold increase in denitrification rates when the moisture content increased from 10 to 15%, probably due to increased mobility of NO_3^-. Reddy et al. (1978) suggested that rates decreased at high moisture contents due to NO_3^- dilution.

1.4.4.2. Temperature

The lower temperature limit has an important influence on the extent of the denitrification process in the field. Various lower limits between 1 and 7°C have been quoted in incubation studies (Nommik, 1956; Bailey and Beauchamp, 1973; Bailey, 1976; Lensi and Chalamet, 1979; Jacobson and Alexander, 1980) but significant rates of denitrification have been measured even at 2-5°C (Sørenson, 1978), at 5°C and by extrapolation at 0°C (Smid and Beauchamp, 1976), and at 2°C (Bremner and Shaw, 1958b). Low temperatures seem to affect NO_3^- reduction least, since at low temperatures a build-up of NO_2^- (Cooper and Smith, 1963) and N_2O (Bailey and Beauchamp, 1973; Bailey, 1976) has been found in soil incubations.

Some workers found an optimum temperature between 60 and 67°C (Keeney et al., 1979; Bremner and Shaw, 1958b; Nommik, 1956) because some denitrifying bacteria are thermophilic and also, at high temperatures, NO_2^- formed from NO_3^- reduction can react chemically with oxidised N groups to form gases (Keeney et al., 1979). Others found optimum rates around 37°C (Stanford et al., 1975c; Garcia, 1974).

Over the range of temperatures experienced in the soil, denitrification rates have been shown to follow the Arrhenius equation

(McKenney et al., 1980b; Nommik, 1956):

$$K = A \exp(-E_A/RT) \qquad 1.17$$

where K is the rate constant

A is a constant

E_A is the activation energy

T is the temperature (^oK)

Stanford et al. (1975c) found lower rates than predicted by Equation 1.17. at low temperatures, indicating a higher activation energy, and possibly therefore a different mechanism.

The ratio of rate constants for a 10^oC temperature difference (Q_{10}) depends on the activation energy for a process. Quoted Q_{10} values for denitrification vary from 1.6 to 3.2 (Nommik, 1956; Jacobson and Alexander, 1980; Lind, 1980; McKenney et al., 1980b).

1.4.4.3. Cycles of Wetting and Drying and Freezing and Thawing

Rewetting dry soil stimulates respiration and organic matter decomposition (Birch, 1958, 1959; McGarity, 1962; Cawse and Sheldon, 1972) since drying increases the availability of organic matter (Soulides and Allison, 1961; McGarity, 1962; McKenzie and Kurz, 1976; Patten et al., 1980) and by killing many existing microbes leads to a new vigorous microbial population (Soulides and Allison, 1961). On drying and rewetting the relative number of nitrifying bacteria increases (Harpstead and Brage, 1958), thus releasing NO_3^- (Cawse and Sheldon, 1972; Soulides and Allison, 1961). The increase in energy source, O_2 demand and NO_3^- due to rewetting dry soil can increase the rate of denitrification up to threefold (Letey et al., 1980c; McKenzie and Kurtz, 1976). The rate of denitrification subsequent to storing soil in a dry condition for 20 weeks instead of 9 days was increased by 35-154% and more than quadrupled by storing at 100^oC instead of 25^oC (Patten et al., 1980).

Freezing and thawing is now known to have a similar effect, although to a lesser extent (Soulides and Allison, 1961), the effect being the greater, the lower the minimum temperature reached (McGarity, Schaefer, 1964).

1.4.4.4. pH

The pH optimum for denitrification is around 8 (van Cleemput and Patrick, 1974; Bremner and Shaw, 1958a). The rate of denitrification decreases with increasing acidity (Wijler and Delwiche, 1954; Bremner and Shaw, 1958b; Cady and Bartholomew, 1960; Ekpete and Cornfield, 1965; Guthrie and Duxbury, 1978), very little denitrification occurring below pH 4.5 (Bremner and Shaw, 1958b; Dubey and Fox, 1974; van Cleemput and Patrick, 1974). Müller et al., (1980) found that much of the variability in denitrification rates (see Table 1.3) was due to pH differences.

Below pH 4.5, NO and NO_2 are often found (Bollag et al., 1972b; Koskinen and Keeney, 1982; Wijler and Delwich, 1954) but this is probably due to the chemical breakdown of NO_2^- in soils rather than true denitrification (Reuss and Smith, 1965; Nelson and Bremner, 1970; Bulla et al., 1970; Balasubramanian and Kanehiro, 1976) and probably occurs in aerobic conditions also (Bollag et al., 1972b).

Low pHs have usually been found to affect N_2O reduction more than NO_3^- reduction, i.e. higher N_2O concentrations are found during incubations of low pH soils (Wijler and Delwiche, 1954; Nommik, 1956; Hauck and Melsted, 1956; Van Cleemput, 1971; Van Cleemput et al., 1975; Cho and Sakdinan, 1978). However, there is evidence that this is true only at high NO_3^- concentrations, i.e. the inhibition of N_2O reduction by NO_3^- is enhanced in acid conditions (Blackmer and Bremner, 1978; Koskinen and Keeney, 1982).

Higher pHs sometimes cause a lag in NO_2^- reduction and therefore a build up of NO_2^- (Cooper and Smith, 1963; Van Cleemput and Patrick, 1974).

1.4.4.5. Organic Matter and Organic Matter Amendments

In incubations where NO_3^- and pH are not the limiting factors, denitrification can often be correlated with organic matter content (Bremner and Shaw, 1958a; Stefanson, 1973; Dubey and Fox, 1974) or some index of available organic carbon, e.g. the glucose equivalent measured in a 0.01M $CaCl_2$ extract (Stanford et al., 1975b), water extractable carbon (Burford and Bremner, 1975; Balasubramanian and Kanehiro, 1976), CO_2 evolution during anaerobic incubation (Lind,

1980) or aerobic incubation (Reddy et al., 1982). Variability in extractable carbon accounted for most of the variation in denitrification rates in soils studies by Stanford et al. (1975b) (see Table 1.2).

Adding glucose to soil usually increases the rate of denitrification (Bremner and Shaw, 1958a; Stanford et al., 1975a; Yoshinari et al., 1977; Jacobson and Alexander, 1980) and the range of rates with an added carbon source (Table 1.3) is greater than without (Table 1.2). For example adding 0.1mg g^{-1} glucose increased the denitrification rate 15 fold in soil from a B horizon studied by Myers and McGarity (1972). As well as providing an energy source for the existing denitrifying population, glucose has been found to increase the population by several orders of magnitude (Ardakani et al., 1975).

Similar effects have been shown with compost (Ekpete and Cornfield, 1965), ground clover (Broadbent, 1951), and sewage sludge and manure (Guenzi et al., 1978; Pomares-Garcia and Pratt, 1978). On the other hand, the addition of organic material of a wide C:N ratio can immobilise added N and reduce denitrification. Denitrification was reduced in a flooded soil by adding rice straw (Patrick and Tusneem, 1972), and by adding ground straw to soil prior to flooding and incubation N losses were reduced by 30% (Patrick and Gotoh, 1974). Such material can also cause fungal growth and a drop in pH, thus reducing denitrification rates (Bowman and Focht, 1974).

There is evidence that when available organic matter is low, NO_3^- is preferentially reduced leading to a build up of NO_2^- (Volz and Starr, 1977), while when readily available organic matter is added more complete reduction to N_2 can occur (Balasubramanian and Kanehiro, 1976; Letey et al., 1980b).

1.4.4.6 Crops

The presence of plants or living roots in incubation experiments increases denitrification rates (Ekpete and Cornfield, 1964; Woldendorp, 1963; Stefanson, 1972b, 1973; Cribbs and Mills, 1979) probably due to the increased O_2 demand, and to root exudates which provide readily available organic matter. Stefanson (1973) found that plants increased the ratio of N_2 to N_2O produced, i.e. they

encouraged more complete reduction. On the other hand, Smith and Tiedje (1979) reported lower denitrification rates in soils with growing plants when NO_3^- concentrations were low, presumably because the plants competed for NO_3^-.

Grassland soils generally have lower NO_3^- concentrations and pHs and there is evidence that denitrification rates are often lower in pasture than in cropped soils, in spite of higher organic matter contents (Burford and Stefanson, 1973; Stefanson, 1976; Terry and Tate, 1980).

1.4.4.7 Type of Fertiliser and Nitrate Concentrations

As would be expected, NO_3^- increases denitrification rates more than NH_4^+ or urea, which must first be nitrified to NO_2^- or NO_3^- for denitrification to take place (Shwartzbeck et al., 1961; Stefanson, 1972c; Craswell, 1979). The relationship between NO_3^- concentrations and rates of denitrification was discussed in Section 1.4.3.

High NO_3^- concentrations inhibit N_2O reduction and cause a build up of N_2O (Blackmer and Bremner, 1976; Balasubramanian and Kanehiro, 1976; Focht et al., 1979; Cho, 1982). Gaskell et al. (1981) showed that this was due to NO_3^- itself and not NO_2^- or NO formed from NO_3^- but Letey et al. (1980c) present evidence that the N_2O accumulation was due to delay in N_2O reductase formation, not the preferential reduction of NO_3^-. The inhibition is enhanced by low pHs (Blackmer and Bremner, 1978). In an acid soil (pH 5.9) N_2O reduction was inhibited even at a NO_3^- concentration of $1\mu g\ g^{-1}$, while in the same soil at pH 7, there was no inhibition, even at $90\mu g\ g^{-1}$ (Terry and Tate, 1980).

1.5. Field Experiments

1.5.1. Nitrous Oxide in the Soil Atmosphere

1.5.1.1. Interpretation

Small increases in N_2O concentrations above ambient concentrations in air ($3.36 \pm 0.16 \times 10^{-7}$ ml ml^{-1} quoted in McKenney et al., 1980b) are now easy to measure by gas chromatography. Measurement of N_2O concentrations in the soil atmosphere are therefore frequently

used to indicate under what conditions and at what depth in the soil denitrification takes place. There are several reasons why these measurements should be interpreted cautiously.

1) Nitrous oxide can be produced by non-denitrifying NO_3^- respirers even though it is not the main product of reduction (Smith and Zimmerman, 1981) and is also produced during nitrification both in pure culture (Yoshida and Alexander, 1970; Ritchie and Nicholas, 1972; Goreau et al., 1980) and in soil incubations (Bremner and Blackmer, 1978, 1979; Freney, 1978, 1979; Smith and Chalk, 1980). At low pHs N_2O may be produced by chemical denitrification (see Section 1.4.4.4).

2) Since N_2O is an intermediate, not the end product, of denitrification (Section 1.3.2) its absence may indicate that N_2O is being reduced more rapidly than it is formed. Some conditions e.g. low temperature, low pH and high NO_3^- concentrations (see Sections 1.4.4.2, 1.4.4.4 and 1.4.4.7) slow down N_2O reduction more than NO_3^- reduction, leading to a build up of N_2O.

3) Where diffusion is slow, e.g. when the moisture content is high, N_2O concentrations can be high even though the rate of N_2O production is low. For example, Webster and Dowdell (1982) showed that although N_2O concentrations in one soil was 80 times that of another, the total flux of N_2O out of the soil, (total N_2O production) was only 14 times greater.

4) Diffusion of N_2O may cause the gas to appear at depths in the soil where no denitrification is taking place (Gilliam, et al., 1978).

5) Replicate measurements of N_2O concentrations may differ by more than a factor of 10 since soils is a heterogeneous medium (Burford and Stefanson, 1973; Dowdell and Smith, 1974; Flühler et al., 1976b), and have a skewed distribution which can be approximately log normal (Burford and Stefanson, 1973; Flühler et al., 1976b; Focht et al., 1979) and it is often difficult to prove differences between treatments. One method sometimes used to show differences has been to calculate cumulative frequency distributions (Flühler et al., 1976a & b; Focht and Stolzy, 1978; Focht et al., 1979).

1.5.1.2. Range of Nitrous Oxide

Concentrations of N_2O in the soil atmosphere have been recorded of up to 8.3×10^{-3} ml ml^{-1} in a California soil at 10cm tension with high NO_3^- concentrations (Rolston et al., 1976) and 2.5×10^{-3} ml ml^{-1} in an Oxford clay soil in England in January (Dowdell et al., 1972). However, concentrations up to 1×10^{-4} ml ml^{-1} are much more common.

At the other extreme, interest has focussed on whether the soil can ever act as a sink for N_2O leading to sub-ambient N_2O concentrations, since N_2O has adverse environmental effects (Section 1.5.4.) Sub-ambient concentrations have been recorded both in incubations (Blackmer and Bremner, 1976) and in field work (Flühler et al., 1976b; Rolston et al., 1978a; Focht et al., 1979) but the analytical techniques of most workers were not sensitive enough to detect such small differences.

1.5.1.3. Relationship between Nitrous Oxide Concentrations and Other Measurements

There is a general trend relating N_2O and O_2 concentrations (see Fig. 1.1) (Burford and Millington, 1968; Dowdell and Smith, 1974; Rolston et al., 1976a, 1978a) unless some other factor, e.g. NO_3^-, is limiting denitrification (Burford, 1976; Focht et al., 1979). The extent of any correlation between N_2O and O_2 has never been statistically investigated.

Since the measured O_2 concentration is that of the macropores while the N_2O originates from anaerobic microsites and diffuses into the macropores, it is possible to obtain high N_2O concentrations even when the measured O_2 concentration is greater than 0.195 ml ml^{-1} (Burford and Millington, 1968; Dowdell and Smith, 1974; Burford et al., 1975).

There is some evidence in the field as in the laboratory (Section 1.4.4.1) that at very low O_2 concentrations N_2O concentrations are low because N_2O is more rapidly reduced (Fig. 1.1 from Dowdell and Smith, 1974). At very low moisture tension diffusion is slower and N_2O remains longer in the soil and is more likely to be reduced (Fig. 1.2 from Focht, 1978).

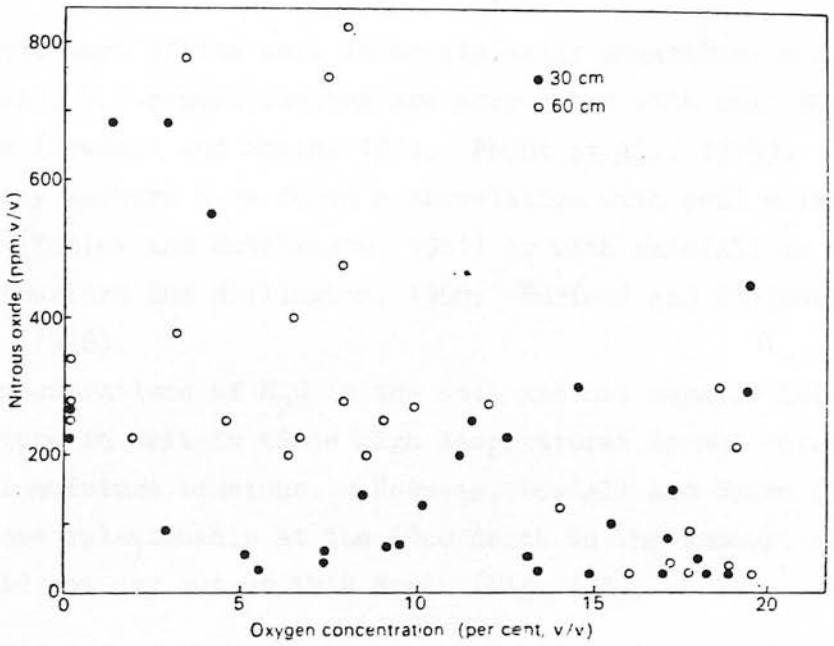


Fig. 1.1. Relationship between O_2 and N_2O concentrations at 2 depths in a clay soil (From Dowdell and Smith, 1974)

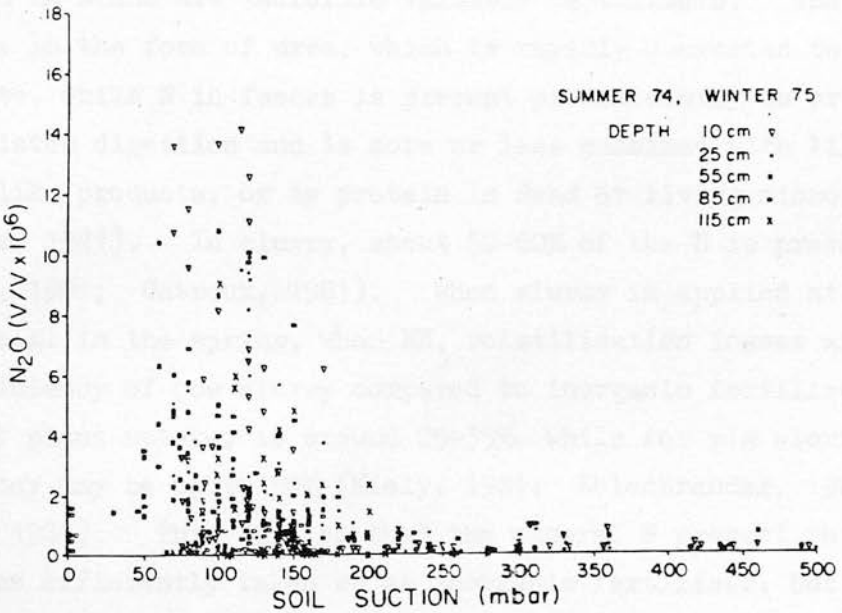


Fig. 1.2. N_2O concentrations in a lysimeter at different depths and as a function of soil suction (June - Sept. 1974 and Jan - April 1975) (from Focht, 1978)

Where much of the soil is consistently anaerobic, e.g. in winter in Britain, N_2O concentrations are correlated with soil NO_3^- concentrations (Dowdell and Smith, 1974; Focht et al., 1979). At other times many workers have found a correlation with soil moisture content (Mosier and Hutchinson, 1981) or with rainfall or irrigation events (Burford and Millington, 1968; Burford and Stefanson, 1973; Burford, 1976).

Concentrations of N_2O in the soil are not usually related to temperature in Britain since high temperatures do not coincide with low soil moisture tensions. However, Dowdell and Smith (1974) found some relationship at the 60cm depth in the summer, in a soil which did not dry out at this depth (Fig. 1.3).

1.5.1.4. The Effect of Manure and Slurry

Farmyard manure and slurry consist of the faeces and urine of animals. The former term is normally applied to the solid mixture formed with bedding material, and the latter to liquid cattle or pig excretia diluted to varying extents with water. Since more than 80% of the N ingested by animals is excreted, slurry and farmyard manure are rich in N and are therefore valuable fertilisers. The N in urine is in the form of urea, which is rapidly converted to ammonium carbonate, while N in faeces is present predominantly as protein which has resisted digestion and is more or less combined with lignin or lignin-like products, or as protein in dead or living microbial cells (Catroux, 1981). In slurry, about 50-60% of the N is present as NH_4^+ (Tunney, 1981; Catroux, 1981). When slurry is applied at optimum times, e.g. in the spring, when NH_3 volatilisation losses are low, the efficiency of cow slurry compared to inorganic fertiliser, in terms of plant uptake, is around 25-35%, while for pig slurry efficiency may be up to 50% (Kiely, 1981; Kolenbrander, 1981a; Tunney, 1981). This implies that the mineral N present in slurry is almost as efficiently taken up as inorganic fertiliser, but that the organic N is much less available.

While it is known that organic N in slurry and manures is mineralised much faster than soil organic matter (Kolenbrander, 1981b) and

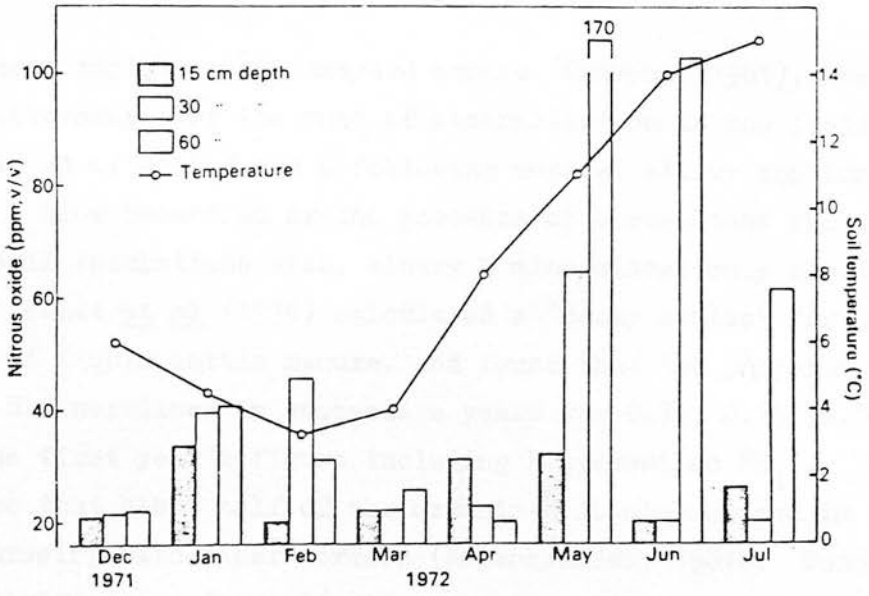


Fig. 1.3. Mean concentration of N_2O in clay soil at 3 depths and the mean soil temperature at 30cm depth (from Dowdell and Smith, 1974)

slurry more rapidly than farmyard manure (Catroux, 1981), there is some controversy over the rate of mineralisation in the field. The build up of soil N and C following several slurry applications implies a slow breakdown or the presence of a resistant fraction, and in soil incubations also, slurry N mineralises only slowly (Catroux, 1981). Pratt et al (1976) calculated a "decay series" for the mineralisation of liquid cattle manure, and found that the proportion of the initial N mineralised in successive years was 0.75, 0.15, 0.10 and 0.05, the first year's figure including N present as NH_4^+. This indicates that about half of the organic-N is mineralised in the first year, agreeing with other workers (Kolenbrander, 1981b; Tunney, 1981). Since nitrification is rapid, by comparison (Germon et al., 1979), the NH_4^+ in slurry initially is more likely to give rise to denitrification than NH_4^+ released from slurry organic matter.

Since slurry and manure are applied to the surface, and NH_4^+ takes part in exchange reactions with soil colloids, release of NO_3^- is at the surface of the soil, and only reaches greater depths by leaching. As Dam Kofoed (1981) has pointed out, appreciable mineralisation and nitrification begin later in the season than crop uptake, but continue later, so that NO_3 thus formed may be available for denitrification over the ensuing winter (Fig. 1.4).

Rolston et al. (1978) applied 34 t ha^{-1} of manure to plots maintained at 1.5 and 7.0 kPa tension by irrigation. They found that at 1.5 kPa N_2O concentrations were slightly higher than in an untreated control plot, while at 7.0 kPa concentrations were over twice as high, even following the application of 300 kg N ha^{-1} as NO_3^-. The effect of the manure was therefore to create more anaerobic conditions, as well as increasing NO_3^- concentrations. Similarly, Focht et al. (1979) found that manure increased N_2O concentrations when NH_4NO_3 fertiliser was also applied. Wallingford et al. (1975) did not add inorganic fertiliser, but found that at high rates of manure application (306 and 687 t ha^{-1} a^{-1}) in irrigated plots N_2O concentrations in the soil profile were increased compared with a control plot during the summer, resulting from increased NO_3^- concentrations, but at a lower rate (58 t ha^{-1} a^{-1}) there was no increase.

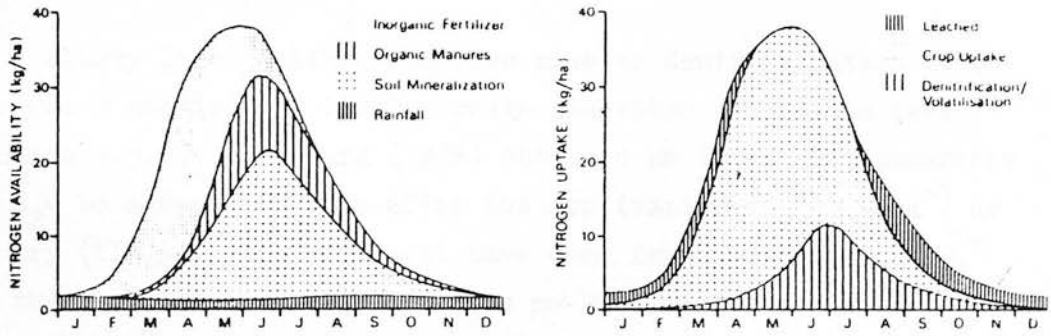


Fig. 1.4. Pattern of availability and uptake of mobile nitrogen over a typical annual cycle.
(from Dam Kofoed, 1981)

Slurry is more likely to give rise to denitrification since the water applied may itself create anaerobic conditions (see Section 1.3.3). Burford (1976) observed an immediate appearance of N_2O to a depth of 40cm after the application of 550 t ha^{-1} as slurry (Fig. 1.5). This must have come from the existing NO_3^- in the soil, since slurry contains no NO_3^-. During the first two months, NO_3^- concentrations fell to almost zero, since the intense reducing conditions favoured denitrification and reduced nitrification either by a direct effect or by inhibition due to gaseous sulphur compounds formed from the slurry. Concentrations of NO_3^- only increased when aeration improved in May allowing nitrification to take place. This increase occurred only in the surface soil, demonstrating that NH_4^+ from the slurry had not been translocated downwards. Correspondingly the initial large concentrations of NH_4^+ in the surface soil had largely disappeared by October. During the summer N_2O concentrations were generally low, but by the winter NO_3^- had leached as far as the 60cm depth, and high N_2O concentrations were found over the winter at depth in the soil. Burford's scheme for the effects of the application is illustrated in Fig. 1.6.

Slurry applied at lower rates, suitable for fertilisation rather than waste disposal, would be unlikely to have such drastic effects on N_2O concentration, since there would be no slurry layer and therefore no long term aeration effects.

1.5.1.5. Nitrous Oxide Concentrations in British Soils

In spite of low temperatures and, often low NO_3^- concentrations, the wet conditions in winter give rise to higher N_2O concentrations than in the summer (Dowdell et al., 1972; Burford, 1976). Looking at several years' data from heavy textured soils Burford et al. (1978a) & b) concluded that high N_2O concentrations persisted for 2 - 6 months during the winter, depending on rainfall. High N_2O concentrations are associated with low soil moisture tensions and tend to increase with depth in the profile (Dowdell et al., 1972; Dowdell and Smith, 1974; Burford, 1976). When warmer temperatures in the late spring increase the O_2 demand and the rate of nitrification

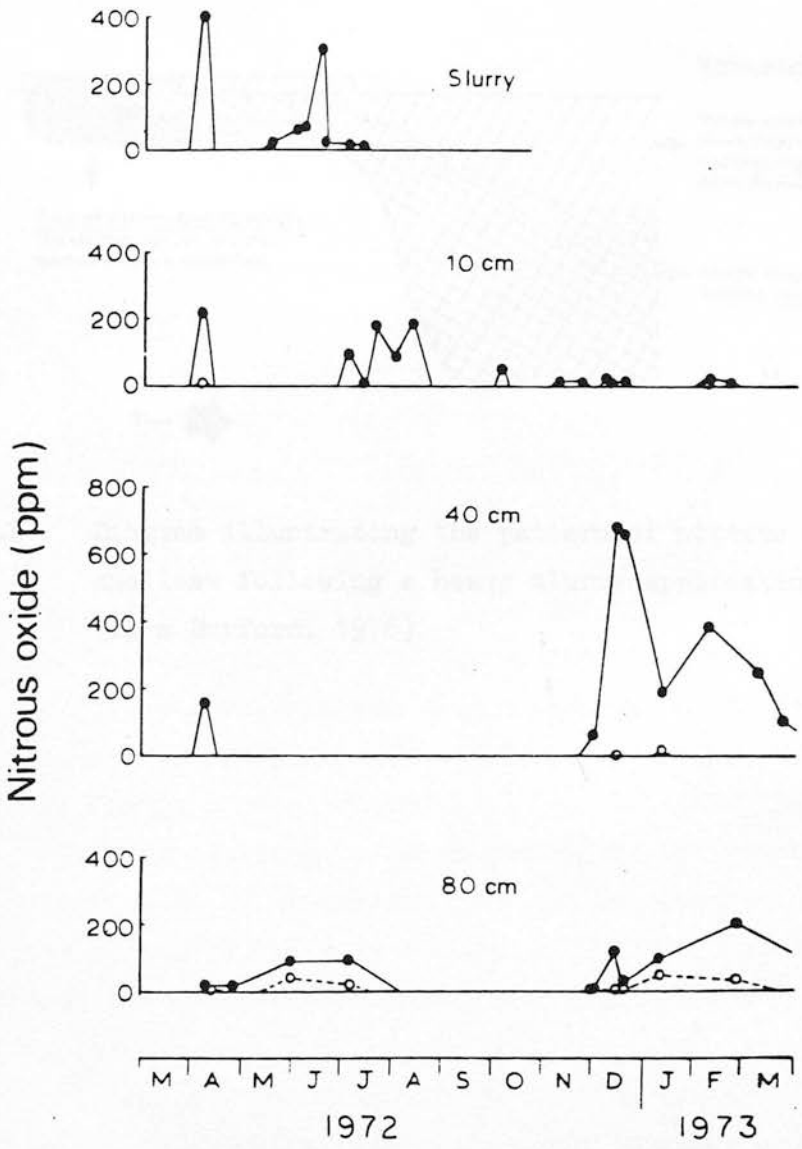


Fig. 1.5. N_2O in the soil atmosphere under the control plot (o—o) and under the heaviest slurry application (●—●) (from Burford, 1976)

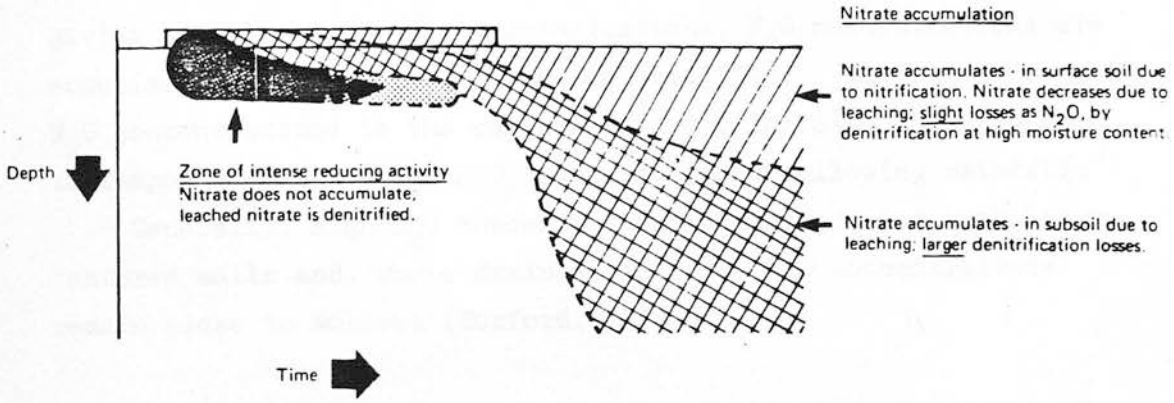


Fig. 1.6. Diagram illustrating the pattern of nitrate accumulation and loss following a heavy slurry application (from Burford, 1976)

giving rise to higher NO_3^- concentrations, N_2O concentrations are sometimes also high (Dowdell and Smith, 1974). During the summer, N_2O concentrations in the surface soil may increase in response to temporary reductions in O_2 concentrations following rainfall.

Generally, high N_2O concentrations are associated with heavy textured soils and, where drainage is good, N_2O concentrations remain close to ambient (Burford, 1978a).

1.5.2. Methods of Measuring N Losses by Denitrification in the Field

The extent of denitrification in the field can be estimated indirectly from the difference between the fertiliser N applied and that recovered from the soil and crop, and leached below the root zone (e.g. Meek et al., 1962; Allison, 1966). When ^{15}N labelled fertiliser is used N immobilised in soil organic matter can also be taken into account (Chichester, 1978; Kowalenko and Cameron, 1978; Jolley and Pierre, 1977; Craswell, 1979; Rolston et al., 1976a, 1979). The assumption that the remaining N has been lost by denitrification is not always valid since N_2O can be lost during nitrification and NH_3 lost by volatilisation, and factors such as run-off may not be accounted for. The large errors in each factor in the difference equation, particularly leaching, mean the final error in the estimation of denitrification is large.

Direct measurement of denitrification involves measuring the flux of N_2O and N_2 gases at the soil surface. However, it is difficult to measure N_2 flux in the presence of ambient concentrations of 0.78 ml ml^{-1} in the atmosphere. Two methods have been devised to circumvent this problem, involving the use of the inhibition of N_2O reduction by C_2H_2 or the use of ^{15}N labelled fertiliser.

Acetylene inhibits N_2O reduction without affecting N_2O production (see Section 1.2.4.1) and therefore in the presence of C_2H_2 all N flux by denitrification is as N_2O. However, it is technically difficult to establish adequate C_2H_2 concentrations in the field. Patriquin et al (1978) used soil covers pushed into the ground and added C_2H_2 to the headspace (0.1 ml ml^{-1}) but this could only have affected the soil enclosed by the walls of the cover. Rolston et al

(1980) and Colbourn and Harper (1982) enclosed the soil by a cover sunk 25cm into the ground and passed C_2H_2 slowly through 1m deep perforated plastic tubes in the soil. Ryden et al. (1979b) allowed C_2H_2 to diffuse radially into the soil from "columns" of C_2H_2 arranged around a soil cover, the C_2H_2 being supplied from probes passed into the "columns", and therefore without causing mass flow. Measured concentrations of C_2H_2 in the soil profile agreed well with concentrations calculated from diffusion theory and even at an air filled porosity of 0.1 ml ml^{-1} C_2H_2 concentrations of 4×10^{-3} ml ml^{-1} were established in the macropores within an hour. Theory would predict that 20 min. is sufficient time for C_2H_2 to diffuse into aggregate centres. Time should then be allowed for the increased N_2O flux to reach an equilibrium (Ryden, 1981).

The inhibition by C_2H_2 of nitrification (Section 1.2.4.4) may lead to lower NO_3^- concentrations and therefore less denitrification. Although Ryden (1981) recommended changing the position of the cover for every measurement, even when this was not done and NH_3 fertiliser was applied, there was close agreement between denitrification measured directly and by difference (Ryden and Lund, 1980a) and in another study NH_4^+ and NO_3^- concentrations in the profile of C_2H_2 treated and untreated areas were not significantly different (Ryden and Dawson, 1982).

Since N_2O is more soluble than N_2, the use of C_2H_2 may underestimate flux if dissolved N_2O leaches from the soil profile (Dowdell et al., 1979b).

In the laboratory, C_2H_2 can eventually become ineffective as an inhibitor (see Section 1.2.4.1) and in the field Rolston et al. (1980) found evidence of microbial adaptation after 4 to 5 weeks, but Ryden and Lund (1980a) found no such effect. In 48h incubations of soil previously repeatedly treated with C_2H_2 in the field, carried out by Ryden and Dawson (1982), C_2H_2 was still effective as an inhibitor. Studies in the laboratory have shown that bacteria exist which are able to use C_2H_2 as a carbon source. However, Ryden and Dawson (1982) found no disappearance of C_2H_2 in their incubations.

The use of ^{15}N enriched fertiliser allows the measurement of N_2O and N_2 flux and has been more widely used than the C_2H_2 method (Dowdell and Webster, 1976; Rolston and Broadbent, 1977; Focht and Stolzy, 1978; Rolston et al., 1978a, 1980). However, the flux of N_2 measured is only that from applied fertiliser and not from native soil N or added organic substances, and large amounts of fertiliser must be used. Limmer et al. (1982) recently published details of a novel approach which circumvented these drawbacks. They flushed the soil enclosed in a double walled microplot with a mixture of He and O_2 and then introduced a spike of ^{15}N labelled N_2 and measured the dilution of the labelled N_2 in the flux out of the soil. However, the flushing process alters the aeration status of the soil.

Since ^{15}N-enriched fertiliser is expensive only small plots can normally be used.

In an experiment using ^{15}N and C_2H_2 methods (Rolston et al., 1980) the measured flux for the two methods differed on any one occasion, although the flux integrated over time was similar; the differences could be accounted for by plot differences, since only one plot was used for each treatment. They suggested that differences were due to a delay in the flux of N_2 compared with N_2O. They estimated that the limit of detection for the ^{15}N method was 0.1-0.2kg N $ha^{-1}d^{-1}$ and for the C_2H_2 method 0.001kg N $ha^{-1}d^{-1}$.

The greater sensitivity, lower cost and greater versatility of the C_2H_2 method give considerable advantages over the ^{15}N method. The four main methods for direct measurement of N fluxes are reviewed below.

1.5.2.1. The Micrometeorological Method

This method uses measurement of the N_2O concentration profile above the ground and vertical wind speed profiles to estimate the flux of N_2O from the ground surface (Thorn, 1975). Since the flux measured is an average over a large area, there is no need for time consuming replication (Hutchinson and Mosier, 1981) but it cannot be used with either the ^{15}N or the C_2H_2 method to measure total

denitrification and is only sensitive to fluxes over 0.5kg N $ha^{-1}d^{-1}$ (Mosier and Hutchinson, 1979).

1.5.2.2. Methods Based on Diffusion Theory

The flux of N_2O can be calculated from the N_2O gradient at the soil surface and the diffusion coefficient, using Fick's 1st Law (Equation 1.4). This is valid only if there is a steady state and no N_2O production in the depth over which the gradient is measured.

The method has been used by Burford and Millington (1968) and Burford and Stefanson (1973), using Shearer, Millington and Quirk's equation (1966) to estimate the diffusion coefficient from total and air-filled porosity, and by Rolston et al. (1976a) who determined the diffusion coefficient directly by a laboratory and field method. It is difficult to measure the concentration gradient accurately because of the spatial variability of N_2O concentrations and the difficulty of determining over what depth the concentration gradient is linear (Kimball, 1978; Rolston, 1978b). Thus although the method involves a minimum of disturbance to a plot and allows measurement of the total denitrification by either the C_2H_2 or ^{15}N method, it has not been widely used.

1.5.2.3. Closed Canopy Method

An area of soil is covered with a canopy, the bottom rim of which is pushed into the soil, allowing gases diffusing out of the soil to build up in the headspace. Variants of the method have been used for measuring fluxes of NH_3 and NO_2, involving absorption of the gases in traps inside the canopy (e.g. Kim, 1973) and to measure the flux of H_2 (Conrad and Seiler, 1979). Flux (F) is calculated from the equation:

$$F = V\Delta C / A t T \times 273 \qquad 1.18$$

(Ryden, 1981)

where: V is the volume under the cover

A is the area of soil enclosed by the cover

ΔC is the concentration change over time, t

T is the temperature of the air within the cover (^{o}K)

The methods allow direct and rapid measurement of very low fluxes without relying on the estimation of other parameters and can be used with either the C_2H_2 or ^{15}N method to measure total denitrification. However, the environment within the canopy differs in several important respects from that of uncovered ground.

For example, the temperature within the canopy may differ from the above-ground temperature of the rest of the plot. Methods to counter this have included shading the canopy (Findlay and McKenney, 1979), using thermal instulation and radiation shielding (Hutchinson and Mosier, 1979), and using a "styrofoam" canopy (Mosier and Hutchinson, 1981). Where the canopy is opaque, temperature differences between the inside and outside of the canopy have been less than 3°C (McGarity and Rajaratnam, 1973; Denmead, 1979a; Matthias et al., 1980).

The "pumping action" of air turbulence on soil gases can cause a flux several times greater than that by diffusion alone in bare soil (Kimball and Lemon, 1971), but under a closed canopy this cannot occur and a period of equilibration is necessary so that N_2O concentrations build up to maintain a similar flux to that of surrounding soil (Mosier and Hutchinson, 1981). Most workers have not taken this into account but Mosier and Hutchinson (1981) included a special vent in the design of their cover to transmit pressure fluctuation without allowing N_2O to escape from the headspace.

The concentration of O_2 in the headspace may fall as O_2 diffuses into the ground and this may lead to more anaerobic conditions. McGarity and Hauck (1969) maintained ambient O_2 concentrations under their cover using polarographic O_2 sensors with a relay operated solenoid to inject O_2 into the cover but other workers have not compensated for this factor.

When N_2O or $^{15}N_2$ build up under the cover, the concentration gradient and flux is reduced (Matthias et al., 1978). Although, as a result, concentrations of N_2O or $^{15}N_2$ then increase in the soil to maintain the flux, this means that a steady state is never achieved (Denmead, 1979a) and the higher N_2O concentrations may increase the rate of N_2O reduction. For this reason measurements should be made as soon as possible after closing the cover, during the period when

the concentration in the headspace is increasing linearly (McKenney et al., 1980a; Denmead, 1979b; Findlay and McKenney, 1979; Webster and Dowdell, 1982).

Attempts have been made to correct the flux measured for this factor. Rolston et al. (1978) used the concentration of N_2O in the headspace, and at 2cm depth in the soil (5 replicates), to estimate the diffusion coefficient, and used this and the concentration at 2cm (assuming it did not change while the cover was in place) to correct the flux. Hutchinson and Mosier (1981) devised an elegant solution, requiring measurements of the N_2O concentration under the cover at 3 times ($t = 0$, t_1, and $2t_1$), and the solution of diffusion equations, to estimate flux at $t = 0$.

Ryden (1981) has estimated that rates as low as 3×10^{-4}kg N ha^{-1} d^{-1} can be measured by this method.

1.5.2.4. Canopy Method Involving Continuous Flow

In the continuous flow method air is drawn through the canopy and the effluent gases are either measured directly, or after trapping in some suitable material, so that concentrations of gases in the headspace of the canopy are close to ambient. This means that continuous monitoring is a possibility (Cochran et al., 1981). The method has not been used to measure the flux of ^{15}N-labelled N_2 since N_2 cannot easily be trapped and concentrations are too low to be measured in the effluent gas stream, but has been used in conjunction with the C_2H_2 method to measure total denitrification (Ryden et al., 1979b, 1980a, 1983).

Denmead (1979a & c) measured the N_2O concentration in the effluent gas directly, using a non-dispersive infra-red gas analyser which can measure N_2O concentrations as low as 1.2×10^{-8} ml ml^{-1}. Ryden has developed a method of trapping N_2O in 5Å (5×10^{-10}m) molecular sieve (m.s.) after removing moisture and CO_2 from the gas stream. The traps were effective for 4 hours at a flow rate of 2×10^4ml h^{-1} for the range of fluxes which occurred. In the laboratory the m.s. was emptied into a flask which was then evacuated, and water was added to release trapped N_2O (Dowdell and Crees, 1974). After allowing 5h

for equilibration, samples from the flask were analysed for N_2O (Ryden et al., 1978, 1979b; Ryden and Lund, 1980a & b; Cochran et al., 1981; Ryden and Dawson, 1982; Ryden, 1983).

One advantage of the continuous flow method is that since the canopy is open, the soil is subject to pressure fluctuations similar to those for soil outside the cover (Ryden, 1981).

The flow of gas through the cover creates a small pressure differential which depends on the flow rate and the inlet size, and can cause mass flow of gases from the soil profile. Kanemasu et al. (1974), measuring CO_2 fluxes, found that internal pressure differing by as little as 0.1 Pa could cause flux differences of almost an order of magnitude, but Ryden et al. (1978) and Denmead (1979a) showed that for pressure deficits below 5×10^{-2} Pa, there was no effect on N_2O flux. Using 0.64cm diameter ports at a flow rate of 2×10^4 ml h^{-1} the pressure deficit was low enough not to affect N_2O flux (Ryden, 1978).

The continuous flow method has the advantage of requiring only one measurement and needing no correction factors.

1.5.3. Results of Field Measurements of Denitrification

The results of some field experiments where N_2O flux and total denitrification have been measured directly are summarised in Table 1.5. In most cases only N_2O flux was measured. Fluxes of up to 7.5kg N $ha^{-1}d^{-1}$ for N_2O and 66kg N $ha^{-1}d^{-1}$ for N_2O plus N_2 have been recorded (Rolston et al., 1980). In one experiment a loss of 223kg N ha^{-1} was recorded (Ryden and Lund, 1980a). In general, large losses occurred during periods of high temperature in soil under irrigation receiving large applications of fertiliser N (e.g. Ryden and Lund, 1980a,b; Rolston et al., 1976a, 1978a, 1980). In a typical British soil, Ryden (1983) showed that losses of more than 0.2kg N $ha^{-1}d^{-1}$ occurred when the soil water content was greater than 20%, the NO_3^- concentration in the top 20cm was more than 5µg N g^{-1}, and the soil temperature at 2cm exceeded 5^oC.

High losses of N by denitrification have been recorded in the summer where the soil moisture tension was held constant by irrigation

Table 1.5. N_2 and N_2O fluxes measured in field experiments

Author	Soil and conditions during Flux measurements	Gas Flux Measured	Max. Flux (kg N $ha^{-1}d^{-1}$)	Total N lost (kg N ha^{-1})
Hall & Burford, 1975	Well drained day loam Closely mown sward, recently fertilized, at field capacity	N_2O	1.92	n.d.
Rolston et al., 1976a	Loam, pasture soil at 10cm tension with 300kg N ha^{-1} as KNO_3 in Nov. Flux over subsequent 30 days	N_2O	0.70	7.8
		N_2 (using ^{15}N)	11.00	126.9
Patriquin et al., 1978	Loam soil planted with maize. Flux measured following urea and ammonia application from June-Sept.	$N_2O + N_2$ (using C_2H_2)	0.40	n.d.
Ryden et al., 1978	Loam soil planted with celery following irrigation and 120 kg N ha^{-1} as $(NH_4)_2SO_4$ in summer	N_2O	0.48	0.72

/...

Table 1.5. contd.- 2

Author	Soil and conditions during Flux measurements	Gas Flux Measured	Max. Flux (kg N $ha^{-1}d^{-1}$)	Total N lost (kg N ha^{-1})
Rolston et al., 1978a	Well drained alluvial soil at constant water content with 300kg N ha^{-1} as NO_3^- during summer with manure $3.4x10^4$kg at 15cm tension	N_2O	7.5	9.9
		N_2(using ^{15}N)	66.0	198
	at 70cm tension	N_2O	3.0	5.4
		N_2(using ^{15}N)	10.0	42.0
	cropped at 15cm tension	N_2O	0.9	4.3
		N_2(using ^{15}N)	4.0	30.0
	at 70cm tension	N_2O	0.4	1.8
		N_2(using ^{15}N)	1.8	7.0
	uncropped at 15cm tension	N_2O	0.5	2.1
		N_2(using ^{15}N)	1.8	5.7
	at 70cm tension	N_2O	0.13	0.6
		N_2(using ^{15}N)	1.1	3.6
Denmead et al., 1979b	Flooded rice soil with 40kg N ha^{-1} as NO_3^-	N_2O	0.38	0.38
		N_2(by difference)	n.d.	27.0

/...

Table 1.5. contd. - 3

Author	Soil and conditions during Flux measurements	Gas Flux Measured	Max. Flux (kg N $ha^{-1}d^{-1}$)	Total N lost (kg N ha^{-1})
Denmead et al., 1979c.	Sandy loam with clay subsoil. Pasture, unfertilised. Flux measured over 5 months from winter to summer.	N_2O	0.22	n.d.
Findlay & McKenney, 1979	Range of soils and management systems in S.W. Ontario in summer.	N_2O	0.053	n.d.
Hutchinson & Mosier, 1979	Irrigated cornfield, following application of 200kg N ha^{-1} as NH_3 during the summer	N_2O	0.41	2.6
McKenney et al., 1980a	Sandy loam with 366kg N ha^{-1} as NH_4NO_3 planted to maize. Flux measured June-Nov.	N_2O	0.024	0.85
	Clay soil with 168kg N ha^{-1} as NH_4NO_3. Flux measured Oct.-Nov. and May-June.	N_2O	6.02	n.d.
Ryden & Lund 1980a	7 sites of irrigated land planted to vegetables. Flux measured June-Feb.	N_2O	0.35	19.6-41.8
		N_2O(using C_2H_2)	3.36	95-223

/...

Table 1.5 contd. - 4

Author	Soil and conditions during Flux measurements	Gas Flux Measured	Max. Flux (kg N $ha^{-1}d^{-}$)	Total N lost (kg N ha^{-1})
Rolston et al., 1980	Loam soil, irrigated with 15% more water than evapotranspiration. Flux following 280kg N ha^{-1} as KNO_3.			
	no straw			
	3 irrigations per week	N_2O	0.32	1.1
		N_2(using ^{15}N)	0.50	3.0
	1 irrigation per week	N_2O	0.08	0.6
		N_2(using ^{15}N)	0.35	2.6
	1 irrigation per 2 weeks	N_2O	0.09	0.3
		N_2(using ^{15}N)	0.05	1.6
	with straw			
	3 irrigations per week	N_2O	2.00	1.8
		N_2(using ^{15}N)	2.25	13.1
	1 irrigation per week	N_2O	1.20	0.8
		N_2(using ^{15}N)	11.40	17.6
	1 irrigation per 2 weeks	N_2O	1.10	1.0
		N_2(Using ^{15}N)	4.10	4.0

/...

Table 1.5. contd. - 5

Author	Soil and conditions during Flux measurements	Gas Flux Measured	Max. Flux (kg N $ha^{-1}d^{-1}$)	Total N lost (kg N ha^{-1})
Ryden, 1983	Grassland loam with with NH_4NO_3 applied in 4 equal amounts. Fluxes measured for 1 yr.			
	Control	N_2O	n.d.	-0.6
		N_2O(using C_2H_2)	n.d.	1.6
	250kg N ha^{-1}	N_2O	n.d.	3.5
		N_2O(using C_2H_2)	n.d.	11.1
	500kg N ha^{-1}	N_2O	n.d.	8.0
		N_2O(using C_2H_2)	n.d.	29.1

n.d. : not determined

in California (Rolston et al., 1978a, 1980) and in an unusually wet English summer (Ryden, 1983). However, in rain-fed agriculture most workers have reported the highest fluxes in spring (Kowalenko and Cameron, 1978; Burford et al., 1978b; Denmead et al., 1979c; McKenney et al., 1980a; Burford et al., 1981; Webster and Dowdell, 1982) or in autumn (McKenney et al., 1980a; Colbourn and Harper, 1982; Armstrong, 1983) i.e. at times when the soil is at low moisture tensions and temperatures warm enough for microbial activity. In winter, fluxes are low (Burford et al., 1978b; Denmead et al., 1979c; Burford et al., 1981; Webster and Dowdell, 1982; Armstrong, 1983; Ryden, 1983) in spite of the fact that N_2O concentrations in the soil profile are usually highest in winter (Section 1.5.1.5). This apparent anomaly is probably because low moisture tensions in the winter lead to low diffusion coefficients, reducing the flux of N_2O. Thus high N_2O concentrations in winter do not imply a high rate of N_2O production in the soil.

The addition of manures or plant residues has been found to increase N flux. In one study, the application of 3.4×10^4 kg ha^{-1} of manure prior to fertiliser addition caused a sixfold increase in denitrification compared to fertiliser alone (Rolston et al., 1978). The incorporation of celery residues gave rise to a doubling of denitrification rates (Ryden et al., 1979b).

Following the application of about 90 t ha^{-1} a^{-1} of pig slurry for a 4 year period Vetter and Steffens (1981) estimated denitrification losses by difference, allowing for leaching and NH_3 volatilisation, and found that 26% of the added N was lost by denitrification. Sanders (1980 - quoted in Dowdell, 1981) measured N_2O emission directly, following the application of ammonium nitrate (65 kg N ha^{-1}) or an equivalent amount of mineral N as slurry (total N 122 kg N ha^{-1}). Fluxes of N_2O were much higher (up to 52 g N ha^{-1} h^{-1}) with mineral fertiliser compared to slurry (up to 5 g N ha^{-1} h^{-1}) and resulted in the loss of 3% of the applied N from inorganic N compared to 0.4% of N applied as slurry.

Fluxes of N_2O and N_2 are usually highest in the first few days following the application of fertiliser if conditions are wet, but

return to background levels after 1-4 weeks (Rolston et al., 1978, 1980; Mosier and Hutchinson, 1981; Webster and Dowdell, 1982; Armstrong, 1983; Ryden, 1983). During the time when NO_3^- concentrations in the soil are high, fluxes are high following irrigation or rainfall (Denmead et al., 1979c; Hutchinson and Mosier, 1979; Mosier and Hutchinson, 1981; Rolston et al., 1980; Ryden and Lund, 1980a & b; Webster and Dowdell, 1982; Ryden, 1983). Background rates for total denitrification in two soils have been given as 10-20 g N ha^{-1} d^{-1} (Ryden, 1982) and for N_2O flux up to 4.8 g N ha^{-1} d^{-1} (Webster and Dowdell, 1982) and 1 g N ha^{-1} d^{-1} (Armstrong, 1983).

It has been shown that fluxes vary diurnally - the flux at midday was 5 times greater than that at midnight (Denmead et al., 1979b). The flux is usually greatest during the afternoon when the temperature is highest (Ryden et al., 1978; Mosier and Hutchinson, 1981).

Since both NO_3^- and N_2O concentrations in the soil have a high spatial variability (see Section 1.5.1.1), it is not surprising that, for fluxes of N_2O, coefficients of variation ranging from 23.7 to 77% have been reported (Ryden and Lund, 1980b; Hutchinson and Mosier, 1979; Mosier and Hutchinson, 1981).

Since, up to now, few studies have measured total denitrification as well as N_2O fluxes, it is difficult to draw conclusions about the ratio of N_2 and N_2O fluxes. From a compilation of about 20 studies CAST (1976) suggested an average ratio of about 16 for denitrification in agricultural soils. In California the ratio of N_2 to N_2O flux in two studies was found to vary from 2.3 to 8.4 (Ryden and Lund, 1980a) and 2.7 to 22 (Rolston et al., 1978, 1980). Higher values of the ratio corresponded to more extreme reducing conditions, e.g. following irrigation (Rolston et al., 1980) and in the autumn, when temperatures were high enough to cause a high O_2 demand, rather than in winter (Ryden and Lund, 1980a). In one study in England in winter, in undrained soil the N_2 to N_2O flux ratio for overall N losses was about 37, whilst in a drained soil of the same series virtually all flux was as N_2O (Dowdell, 1982). Colbourn and Harper (1981) found flux ratios of 7-10 in their study. In an English grassland soil with low NO_3^- concentrations and a moisture content greater than

0.25ml g^{-1} the ratio was above 5.7 while at high NO_3^- concentrations lower ratios were usually recorded (Ryden, 1983). He attributes this to the inhibitory effect of NO_3^- on N_2O reduction (Section 1.4.4.7).

There has been one report of a "negative flux" (0.6kg N ha^{-1} a^{-1}), i.e. a net absorption of N_2O by the soil. This was for a plot which had received no fertiliser (Ryden, 1983).

1.5.4. Summary and Implications of the Results of Field Experiments

The experiments reviewed above have shown that ideas developed in laboratory studies are valid also in the field. They have demonstrated the extent of denitrification losses, and begun to highlight some of the management practices affecting losses.

As yet, few studies have investigated losses of N by denitrification directly in British soils and none at all in Scottish soils. However, many U.K. studies indicate an average take-up of around 50% of added fertiliser by the crop (Dowdell, 1982). Given that the soil organic N content is largely constant, this would indicate that large losses of N are taking place either by leaching or denitrification.

In natural ecosystems, losses of N by denitrification are likely to be small, since under such systems NO_3^- concentrations tend to be low - i.e. the N cycle is largely a 'closed cycle' with N circulating between plants and soil. Modern agricultural practices involving the addition of large amounts of industrially fixed N have created a much more open cycle. This represents not just a wasteful use of energy and money but also a potential environmental hazard, since N_2O is known to destroy ozone in the upper atmosphere which gives protection from harmful ultra-violet radiation (Crutzen, 1974; McElroy *et al.*, 1976, 1977; Liu, 1977) and may contribute to the so-called "greenhouse effect" (Wang *et al.*, 1976). The further development of methods to quantify denitrification losses, and management practices to minimise losses, is therefore of great importance.

1.6. Introduction to Experimental Work

Since there is now a considerable literature on denitrification

in laboratory experiments, it was decided in this study to concentrate on field based experiments. Initially concentrations of O_2 and N_2O were measured in a soil profile over a period of 2 years as an indicator of the occurrence of denitrification. Since a high degree of replication was required, it was possible to use only one field site, and an imperfectly drained pasture soil was chosen as being typical of soils of the Eastern lowlands of Scotland. The study incorporated the use of both inorganic and organic fertiliser (as calcium nitrate ($Ca(NO_3)_2$ and cow slurry respectively) as well as control plots receiving no fertiliser, with applications at various times of the year. This enabled a qualitative comparison to be made of the treatments used as well as showing when denitrification was most likely to take place.

By measuring gas diffusion coefficients in the soil, an estimate could be made of the likely flux of N_2O during the two years for which data were collected. This did not allow the calculation of denitrification from N_2 fluxes.

Supporting laboratory experiments were also carried out to measure rates of NO_3^- and N_2O reduction in soil from the field site.

During the course of the experimental work, reports of new techniques to measure fluxes of N_2 and N_2O by using C_2H_2 inhibition of N_2O reduction and ^{15}N labelled fertiliser began to appear in the literature. An experiment was therefore carried out using the C_2H_2 technique to measure fluxes of N_2 and N_2O in enclosed microplots. Prior to setting up the field experiments, the C_2H_2 technique was evaluated in the laboratory. During the course of this work it became clear that C_2H_2 had effects on soil respiration. Although this was not directly related to denitrification, it was felt that the process warranted further investigation since it had not been widely reported. Some experiments were subsequently carried out to find out whether these effects were due to C_2H_2 consuming organisms, and to isolate such organisms.

Fluxes of N_2 and N_2O from the field site were measured over a 13 month period, during which applications of inorganic fertiliser and slurry were made at intervals. At the same time O_2 and N_2O concentrations in the soil profile were measured.

2. PRELIMINARY FIELD EXPERIMENT

The preliminary field experiment was carried out during the autumn, winter and spring of 1978/79 for several purposes. It was used to establish suitable methods of collection and analysis of N_2O, O_2, and CO_2 in the soil profile and to obtain some information on the concentrations of the gases at different depths and following treatments with different sources of N. The frequency distributions of the gas concentrations were examined in view of published reports of non-normal distributions, particularly of N_2O (see Section 1.5.1. 1.). The experiment also served the purpose of assessing at which time of the year, and at which depth, the highest N_2O concentrations were to be found in the soil profile and how concentrations varied with measured O_2 and CO_2 concentrations. Available nitrogen in the profile and nitrogen uptake by the herbage were also measured.

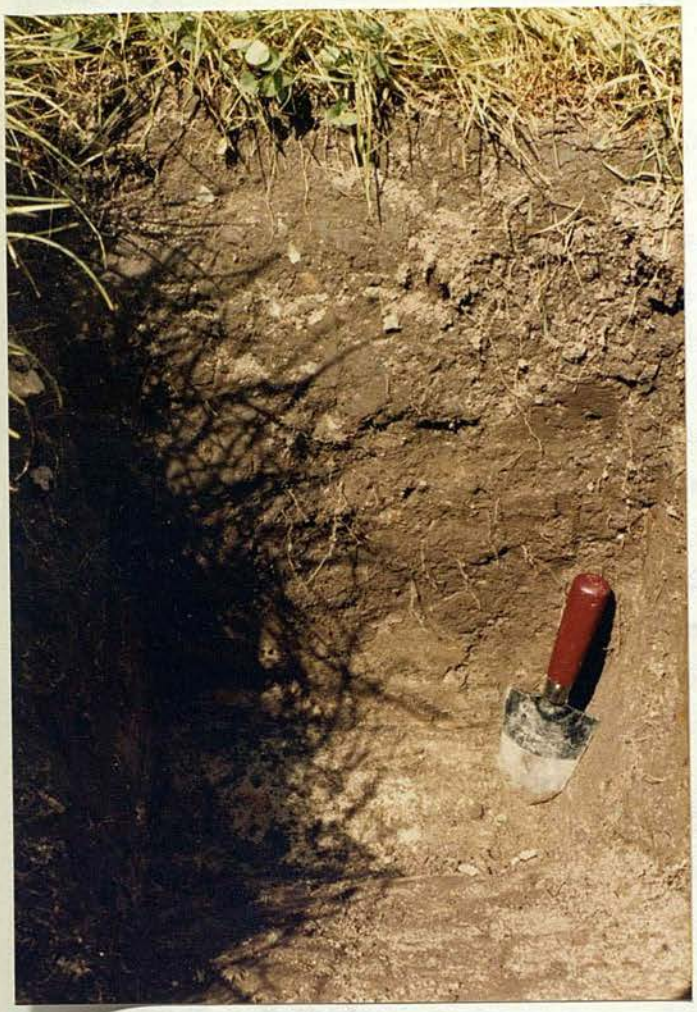
The information gained was used to design a suitable experiment for the following season.

2.1. Field Site

The site chosen was part of a field at Langhill Farm, near Roslin, Midlothian at a height of 168m (500') above sea level. The area experiences an annual average rainfall of 905mm (Meteorological records for the Bush station which is within two miles of the site and at the same height above sea level).

A pasture consisting of perennial ryegrass and wild white clover had been sown in 1975 and subsequently the grass had been used for grazing and as a hay crop. Tile drains had been installed before 1950 at greater than the 60cm depth and ran across the field plot.

The soil was of the Macmerry soil series, an imperfectly drained soil of the Winton Association (a stagnogleyic brown earth, according to Avery's (1980) classification). The parent material was a partially sorted till of carboniferous sediments overlying a clay loam till. The profile description below is taken from the Soil Survey Memoirs (Ragg and Futty, 1967) and is typical of the field site. (Plate 2.1)



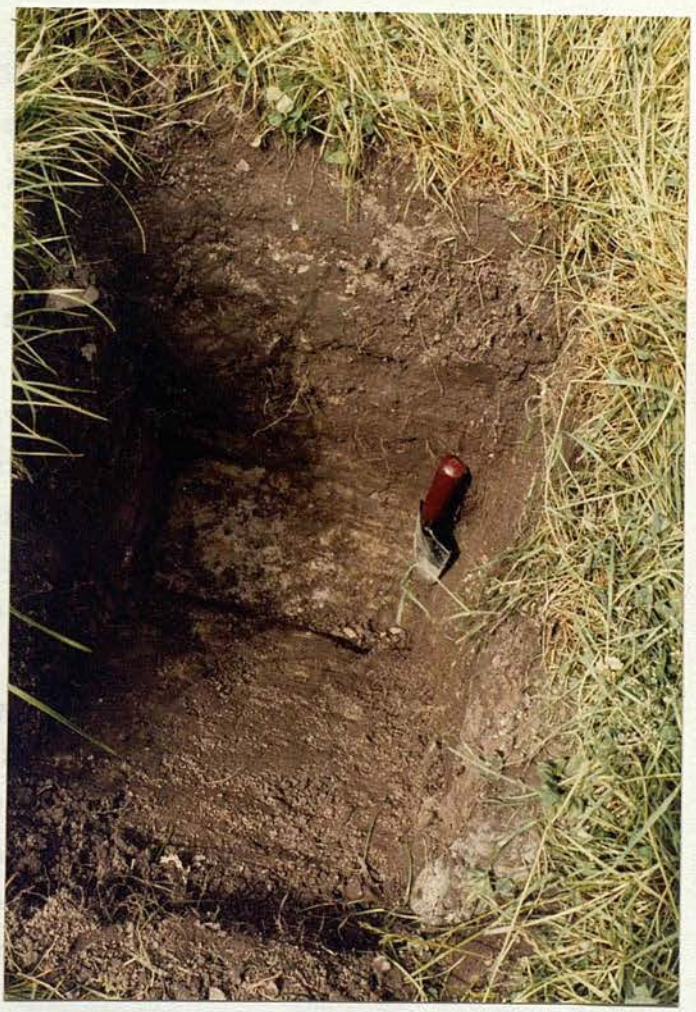


Plate 2.1. Soil profiles from the field plot

S	0-30cm	Dark grey brown (10YR 4/2) sandy loam; medium blocky; friable; mod. organic C; abundant roots; occasional stones; no mottles; clear change into
$B_{2(g)}$	30-51cm	Yellowish brown (10YR/ 5/4) sandy loam; coarse blocky; weakly indurated; firm; occasional roots; frequent stones; prominent strong brown (7.5YR 5/8) mottles, coarse pinkish grey (5YR 6/2) streaks; occasional black manganiferous concretions; sharp undulating change to
$B_{3(g)}$	51-76cm	Reddish brown (5YR 5/4) clay loam; coarse prismatic; plastic; roots rare; frequent stones; frequent strong brown (7.5YR 5/8) and pinkish grey (5YR 6/2) mottles and black manganiferous concretions; gradual change into
$C_{(g)}$	76cm+	Reddish brown (5YR 5/4) clay loam; massive; firm; stones; faint strong brown (7.5YR 5/8) mottles and few grey (5YR 6/1) streaks; manganiferous staining.

2.2. Methods

A 30m x 14m site was chosen in a part of the field with no gradient, and enclosed by a fence to keep out stock. Preliminary analysis of the soil from the plot is given in Table 2.1. Three plots of 10m x 8m were laid out within the site, allowing 1m between the plots. Within each plot 12 soil atmosphere sampling probes (see Appendix 1) were installed in 4 rows of 3 probes, with one probe in each row randomly allocated to each of 3 depths: 15, 30 and 45cm (Fig. 2.1).

On 4th September 1978 a large application of slurry, 273 l m^{-2} (for analysis see Table 2.2) was made to Plot 1. Inorganic nitrogen fertiliser, as calcium nitrate, was applied to Plot 2 on 5th September at a rate equivalent to 100kg N ha^{-1} (Appendix 4).

Gas samples were taken on the first, third and seventh day following the application of slurry and fertiliser and thereafter weekly (Appendices 1-3).

On 25th September and 12th June 8 cores were taken from each plot and ammonium (NH_4^+) concentrations were determined in samples from the

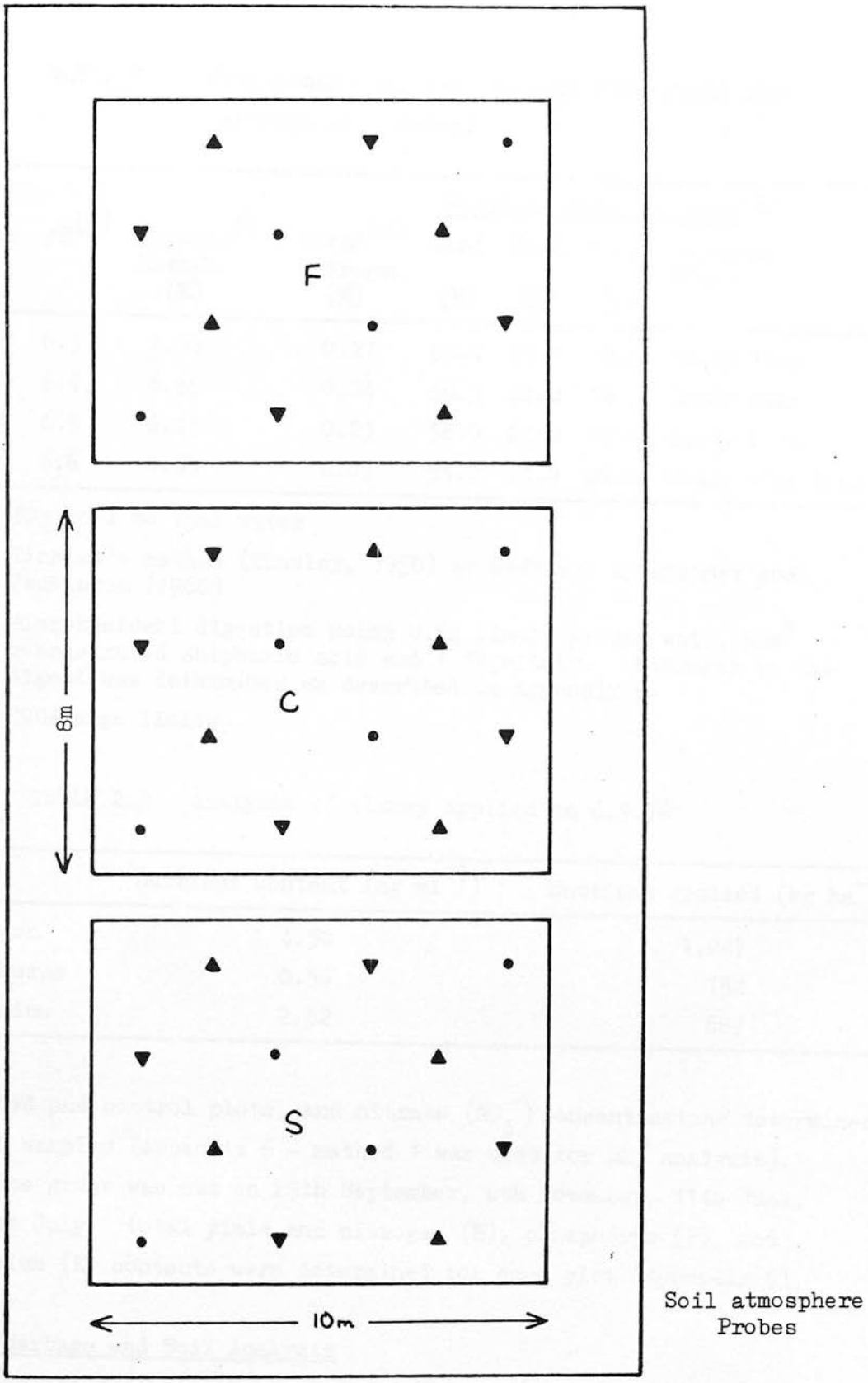


Fig. 2.1. Layout of preliminary field experiment

Table 2.1 Preliminary analysis of soil from field plot (average of 6 cores)

Depth (cm)	pH[a]	Organic Matter (%)[b]	Total Nitrogen (%)[c]	Particle Size Analysis[d] Sand (%)	Silt (%)	Clay (%)	Textural Class
0-10	6.3	7.06	0.27	58.4	22.1	19.5	Sandy loam
10-20	6.4	6.55	0.24	58.5	24.0	18.5	Sandy loam
20-30	6.5	6.24	0.23	58.0	23.0	19.0	Sandy loam
30-40	6.6	1.55	0.07	54.0	24.0	22.0	Sandy clay loam

(a) 30g soil to 75ml water

(b) Tinsley's method (Tinsley, 1950) as modified by Bremner and Jenkinson (1960)

(c) Microkjeldahl digestion using 0.5g finely ground soil, $5cm^3$ concentrated sulphuric acid and 1 "Kjeltab". Nitrogen in the digest was determined as described in Appendix 5.

(d) USDA size limits

Table 2.2 Analysis of slurry applied on 4.9.78

	Nutrient Content ($mg\ ml^{-1}$)	Nutrient Applied ($kg\ ha^{-1}$)
Nitrogen	4.50	1,227
Phosphorus	0.56	152
Potassium	2.52	687

slurried and control plots, and nitrate (NO_3^-) concentrations determined in all samples (Appendix 6 - method 1 was used for NH_4^+ analysis).

The grass was cut on 25th September, 6th November, 11th June, and 2nd July; total yield and nitrogen (N), phosphorus (P), and potassium (K) contents were determined for each plot (Appendix 5).

2.3. Herbage and Soil Analysis

Herbage analysis (Table 2.3 and Fig. 2.2) showed increased yields in both the fertilised and slurried plots in the two months following the application. By 6th November, assuming inorganic fertiliser did

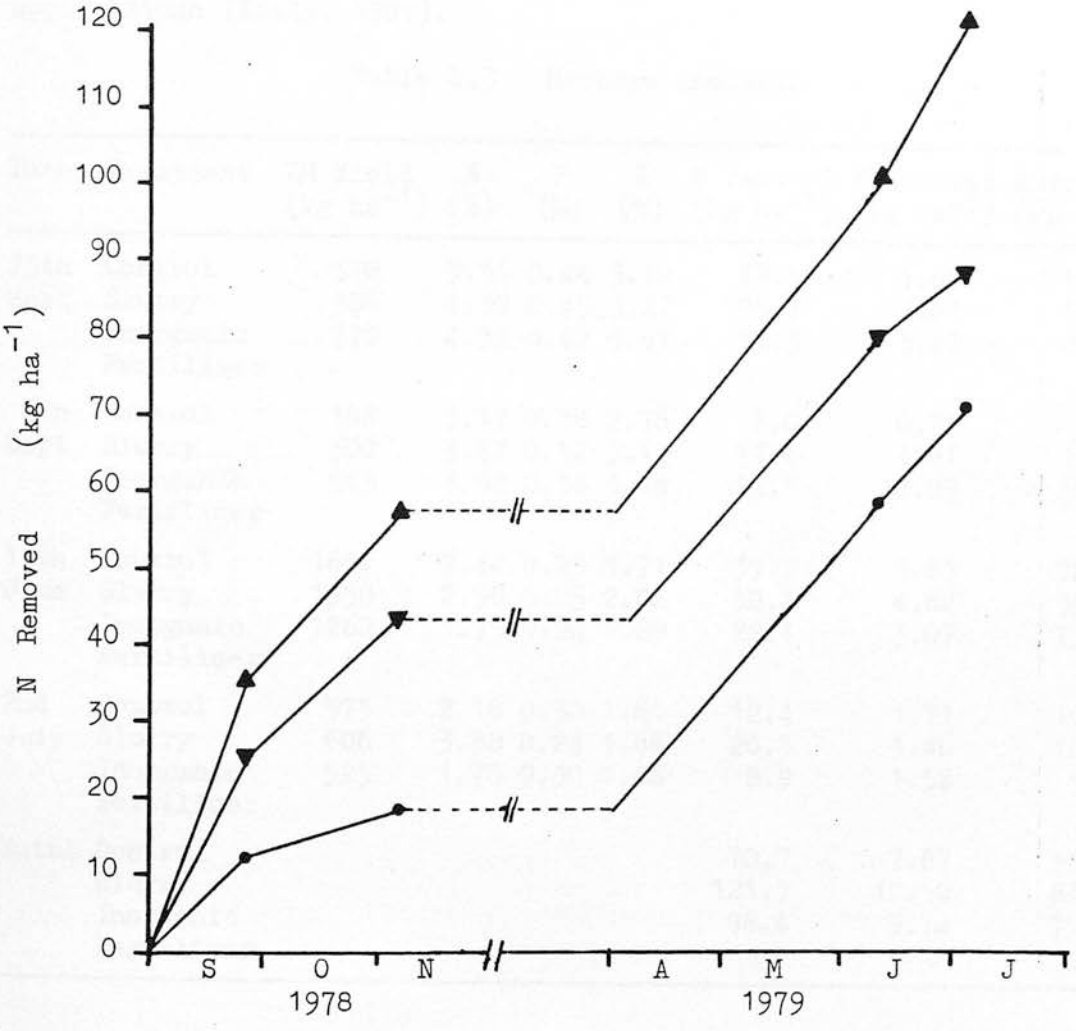


Fig. 2.2. Nitrogen recovered in herbage (cumulative)

▲——▲ fertilised ▼——▼ slurried ●——● control

not affect soil N uptake, 40% of the applied N was taken up by the grass. The slurry continued to increase the yield of herbage in 1979. The proportion of applied N from slurry recovered in the herbage (4.1%) was small compared to quoted percentage recoveries (15%) for slurry applied at optimum times (Kiely, 1981; Tunney, 1981). However, recovery rates for applications much larger than the recommended rate of 30-40 $t\,ha^{-1}$ (Tunney, 1981) have usually been low (Spallacci, 1981), and are lower for autumn or winter applications (Kiely, 1981).

Table 2.3 Herbage analysis

Date	Treatment	DM Yield (kg ha^{-1})	N (%)	P (%)	K (%)	N removed (kg ha^{-1})	P removed (kg ha^{-1})	K removed (kg ha^{-1})
25th Sept	Control	370	3.36	0.44	3.12	12.4	1.62	11.5
	Slurry	586	4.39	0.45	3.27	25.7	2.61	19.2
	Inorganic Fertiliser	770	4.59	0.42	3.57	35.3	3.20	27.5
6th Sept	Control	188	3.17	0.38	2.76	6.0	0.71	5.2
	Slurry	500	3.47	0.32	3.13	17.4	1.61	15.7
	Inorganic Fertiliser	563	3.92	0.34	3.18	22.1	1.89	17.9
11th June	Control	1637	2.44	0.23	1.71	39.9	3.83	28.0
	Slurry	1950	2.98	0.25	2.02	58.1	4.82	39.4
	Inorganic Fertiliser	1262	1.75	0.24	1.89	22.1	3.07	23.9
2nd July	Control	575	2.16	0.30	1.81	12.4	1.71	10.4
	Slurry	606	3.38	0.24	1.66	20.5	1.46	10.1
	Inorganic Fertiliser	525	1.70	0.30	1.86	8.9	1.58	9.8
Total	Control					70.7	7.87	55.1
	Slurry					121.7	10.50	84.4
	Inorganic Fertiliser					88.4	9.74	79.1

Soil analysis showed that three weeks after the slurry application, NH_4^+ concentrations even in the surface soil were low, in contrast to the results of Thijell and Burford (1975) who found high NH_4^+ concentrations in the surface soil for several months following a large slurry application (Table 2.4). This would either indicate very rapid nitrification, or rapid leaching of the liquid fraction of slurry through almost saturated soil. The high NH_4^+ concentration at the 40cm + depth and increased NO_3^- concentrations to a depth of 40cm indicate rapid movement of the slurry. The slightly higher NO_3^-

concentrations in the slurried plot than in the control, observed in samples taken the following summer, were probably caused by mineralisation of organic N from the slurry.

Table 2.4 Soil analysis: Exchangeable Ammonium

Depth (cm)	Exchangeable NH_4^+ - N in samples ($\mu g\ N.g^{-1}$)			
	25th September 1978		12th June 1979	
	Control	Slurried	Control	Slurried
0-10	3.7	2.2	2.2	1.9
10-20	1.8	5.2	1.3	5.2
20-30	0.8	7.2	1.8	0.8
30-40	2.3	7.1	2.4	1.8
40+	1.9(av of 6)	28.5(av of 5)	0.4(av of 6)	0.8(av of 2)

All data are the averages of 8 cores except where stated

Table 2.5 Soil Analysis: Nitrate

Depth (cm)	NO_3^- - N in samples ($\mu g\ N\ g^{-1}$)					
	25th September			12th June 1979		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised
0-10	48.0	1.4	93.5	7.1	12.4	9.5
10-20	16.7	45.3	34.1	7.0	12.3	7.7
20-30	3.4	31.8	21.7	3.9	6.8	3.0
30-40	2.3	16.3	2.4	2.0	3.7	1.4
40+	2.1 (av of 4)	7.3 (av of 5)	0.6 (av of 6)	1.2 (av of 6)	2.3 (av of 2)	1.0 (av of 7)

All data are the average of 8 cores except where stated

Three weeks after the application of inorganic fertiliser, NO_3^- concentrations were intermediate between those of the control and slurried plots but by the following June there was essentially no difference between NO_3^- in the control and fertilised plots.

2.4. Soil Temperature and Rainfall

The data presented in Fig. 2.3 are taken from meteorological records at Bush estate recording station.

Rainfall was high in November and from mid December until the end of January, and again during March and April. Soil temperatures were consistently below 5°C at all three depths from the end of

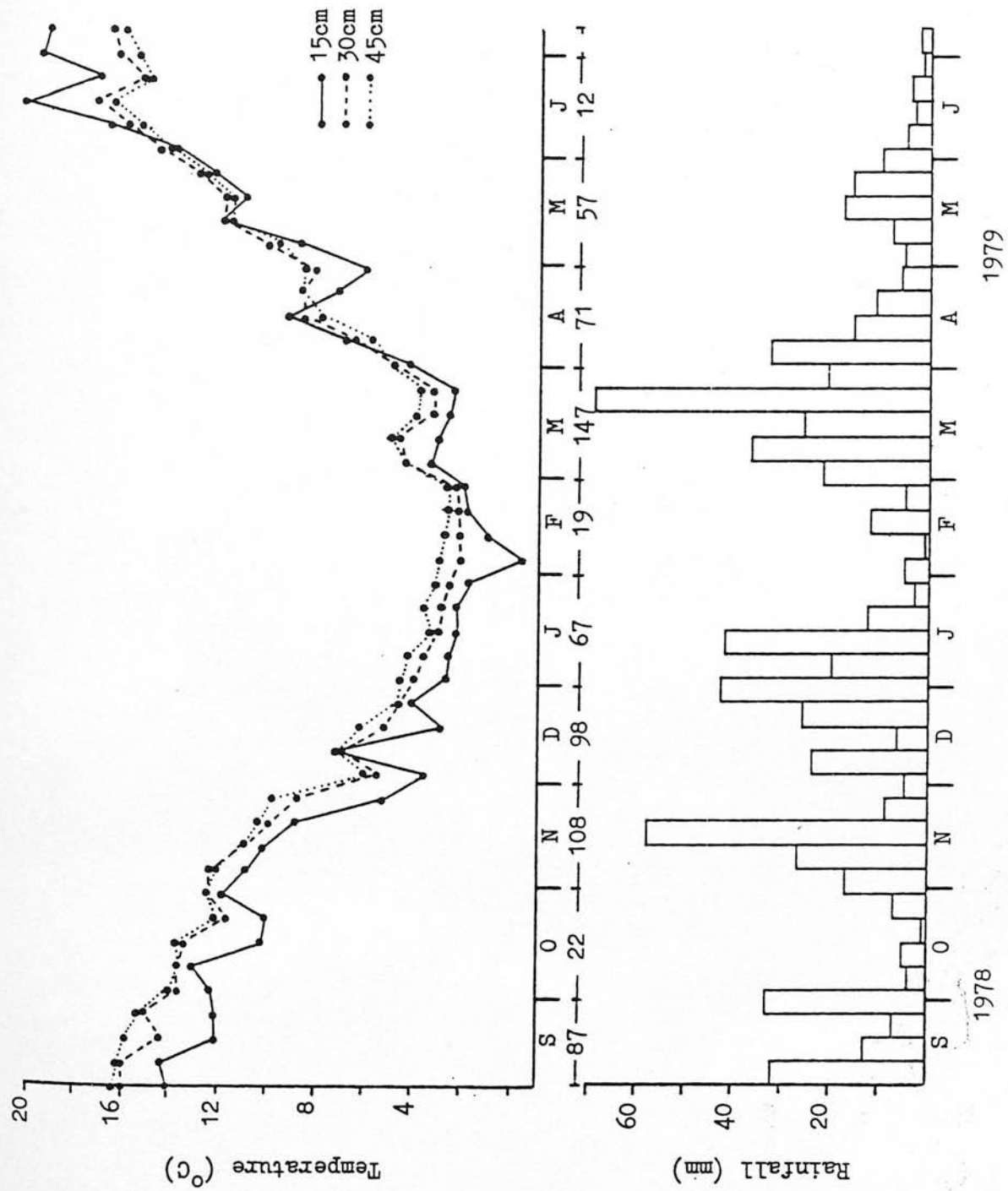


Fig. 2.3. Soil temperatures at the 15, 30 and 45cm depth and weekly and monthly rainfall

November until mid-April. At 15cm the ground was frozen during the whole of February.

2.5. O_2 and CO_2 Concentrations

In all three treatments median O_2 concentrations were about 0.175ml ml^{-1} (Fig. 2.4). However, modal values were very different for the three treatments: 0.180 - 0.185, 0.170 - 0.175 and 0.200 - 0.205ml ml^{-1} for the control, slurried and fertilised plots respectively, and there were fewer low values for O_2 in the control plot.

There was no evidence of a bi-modal distribution as has been observed by some workers (Dowdell et al., 1979; King, 1982).

The data were not normally distributed but a reasonable approximation to a normal distribution (Figs. 2.5 and 2.6) was given by the transformation:

$$X' = \ln (24 - X) \qquad 2.1$$

where X is the O_2 concentration (ml ml^{-1} x 10^2)
X' is the transformed O_2 concentration

The median values for CO_2 concentration (Fig. 2.7) were 0.026, 0.022 and 0.015ml ml^{-1} for the control, slurried and fertilised plots respectively, i.e. the highest value was in the control plot. The data was not normally distributed but could be transformed to give an approximately normal distribution (Figs. 2.8 and 2.9) by the relationship:

$$Y' = \ln (1 + Y) \qquad 2.2$$

where Y is CO_2 concentration (ml ml^{-1} x 10^2)
Y' is the transformed CO_2 concentration

The transformed data for O_2 and CO_2 were used to calculate means of replicate probes on each sampling occasion, and the reverse transform of the mean was used in Figs. 2.10 - 2.12. For the sake of clarity, the points on the Figures are joined but it is recognised that fluctuations between sampling occasions are likely to have occurred.

Generally, peaks in CO_2 corresponded with troughs for O_2, but CO_2 concentrations were less than the corresponding O_2 deficits, for reasons discussed in Section 1.3.3. From week to week CO_2 varied less than O_2, perhaps because dissolved CO_2 had a 'buffer' effect, CO_2 coming out of solution if concentrations in the gaseous phase decreased and dissolving when they increased.

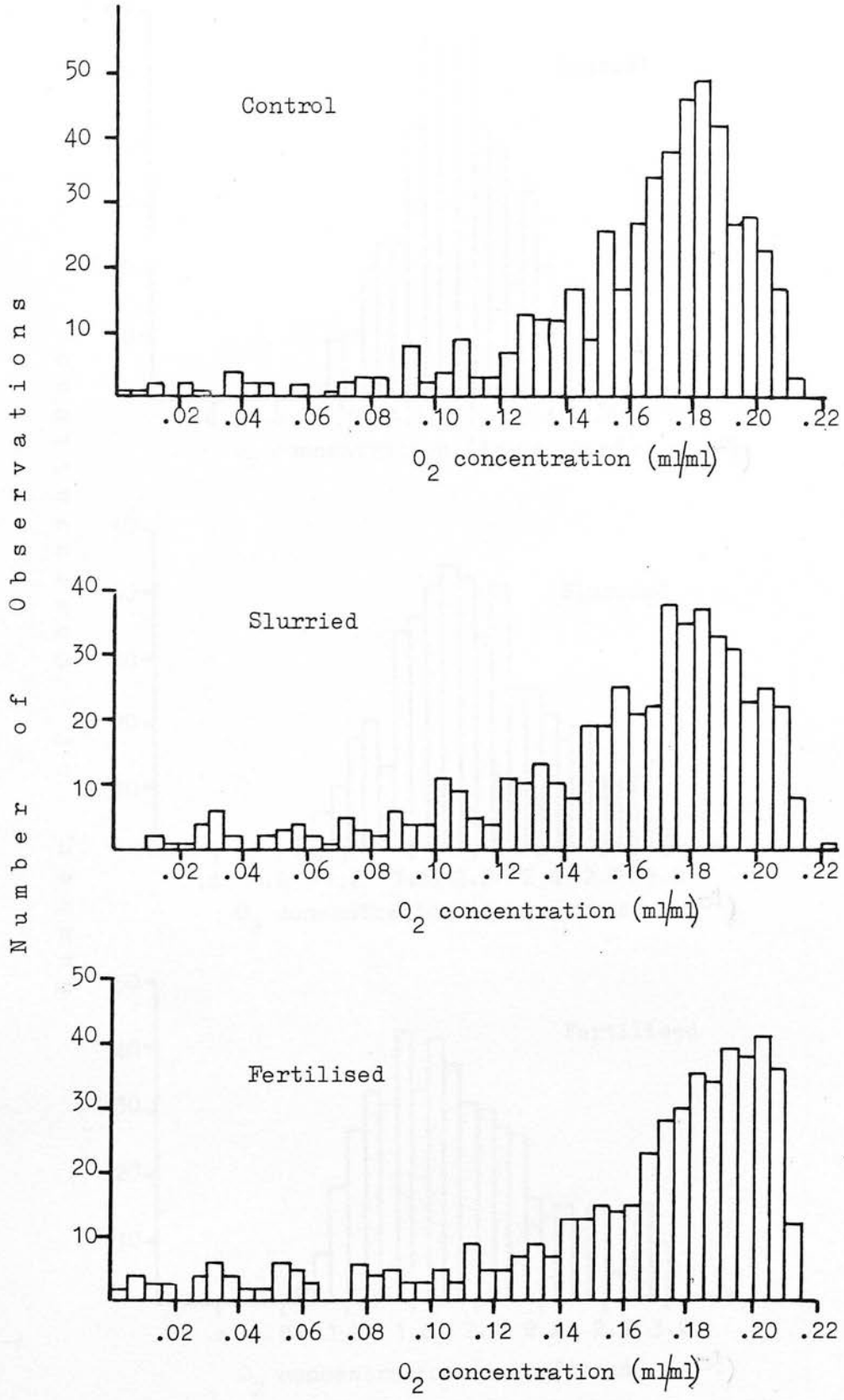


Fig. 2.4. Frequency distributions for untransformed O_2 data

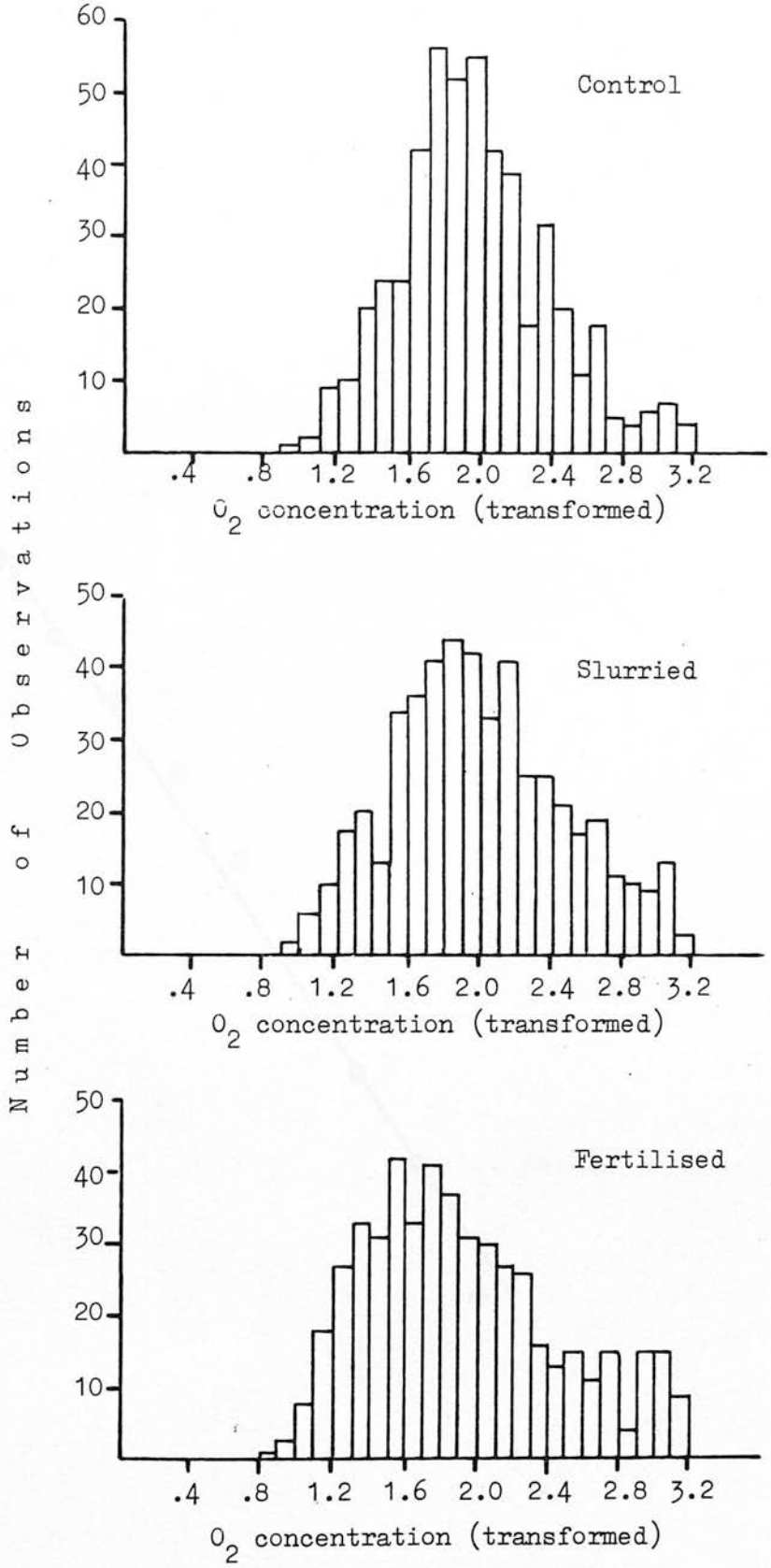


Fig. 2.5. Frequency distributions for transformed O_2 data

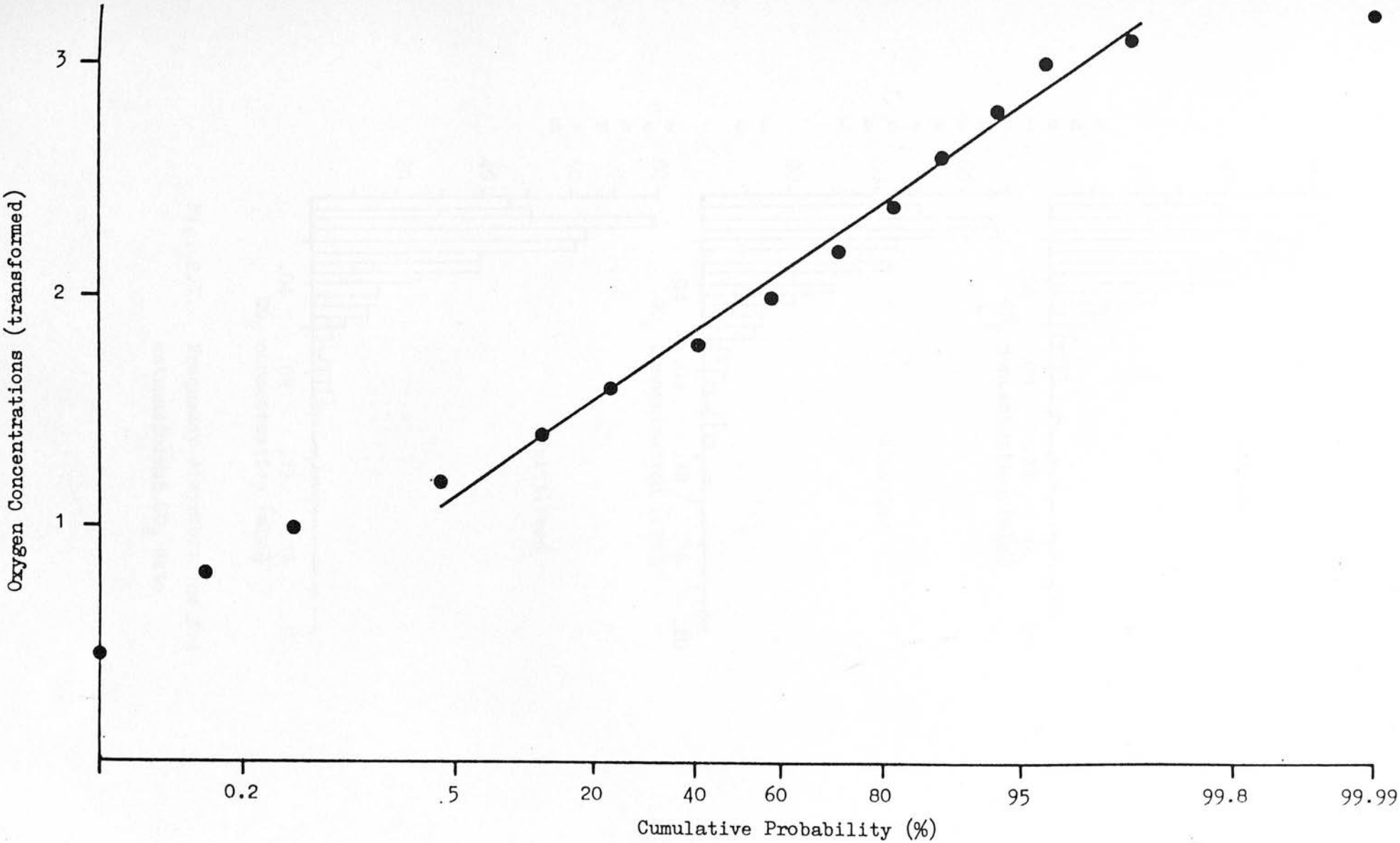


Fig. 2.6. Probability plot for combined O_2 data (transformed)

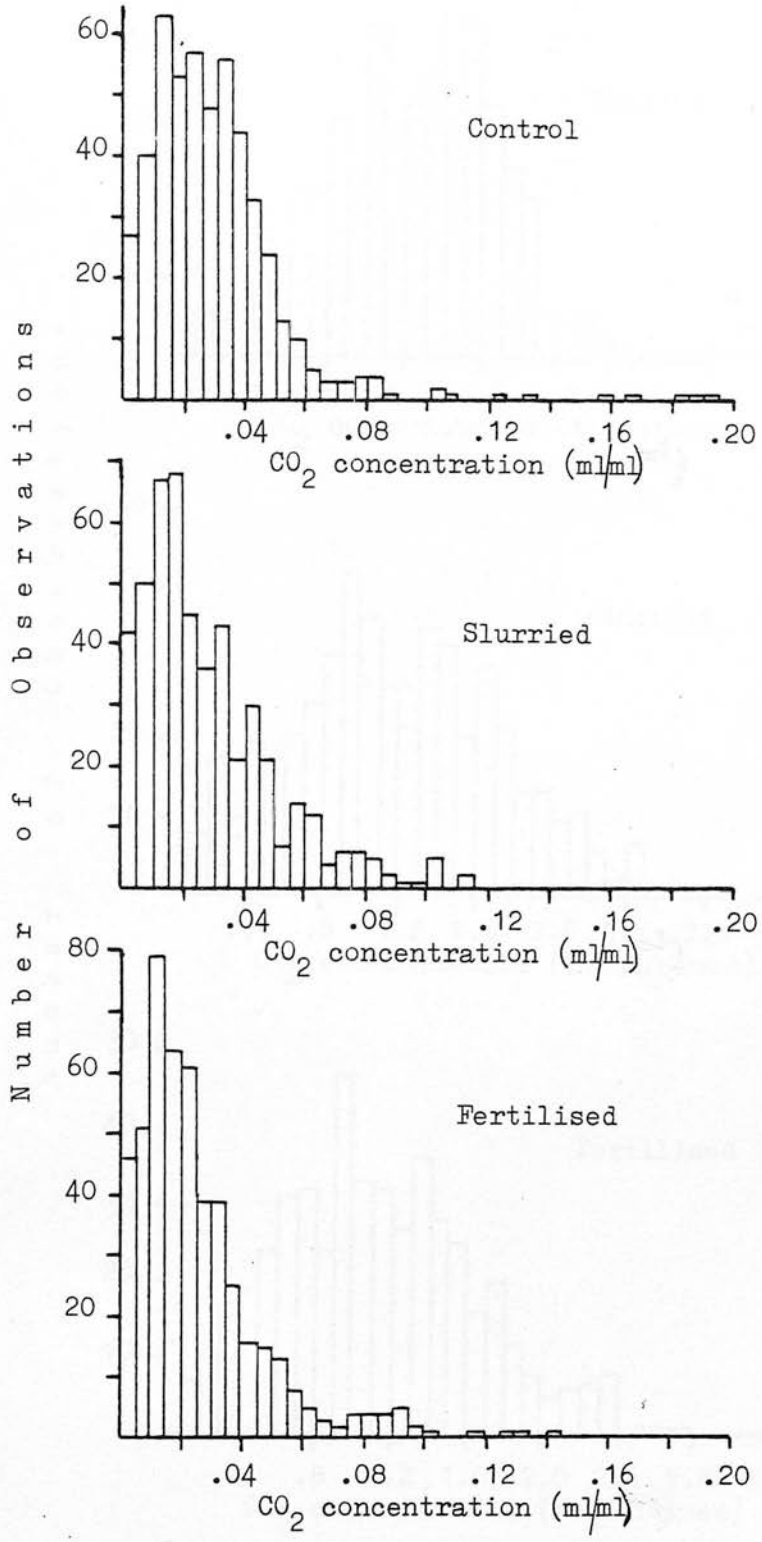


Fig. 2.7. Frequency distributions for untransformed CO_2 data

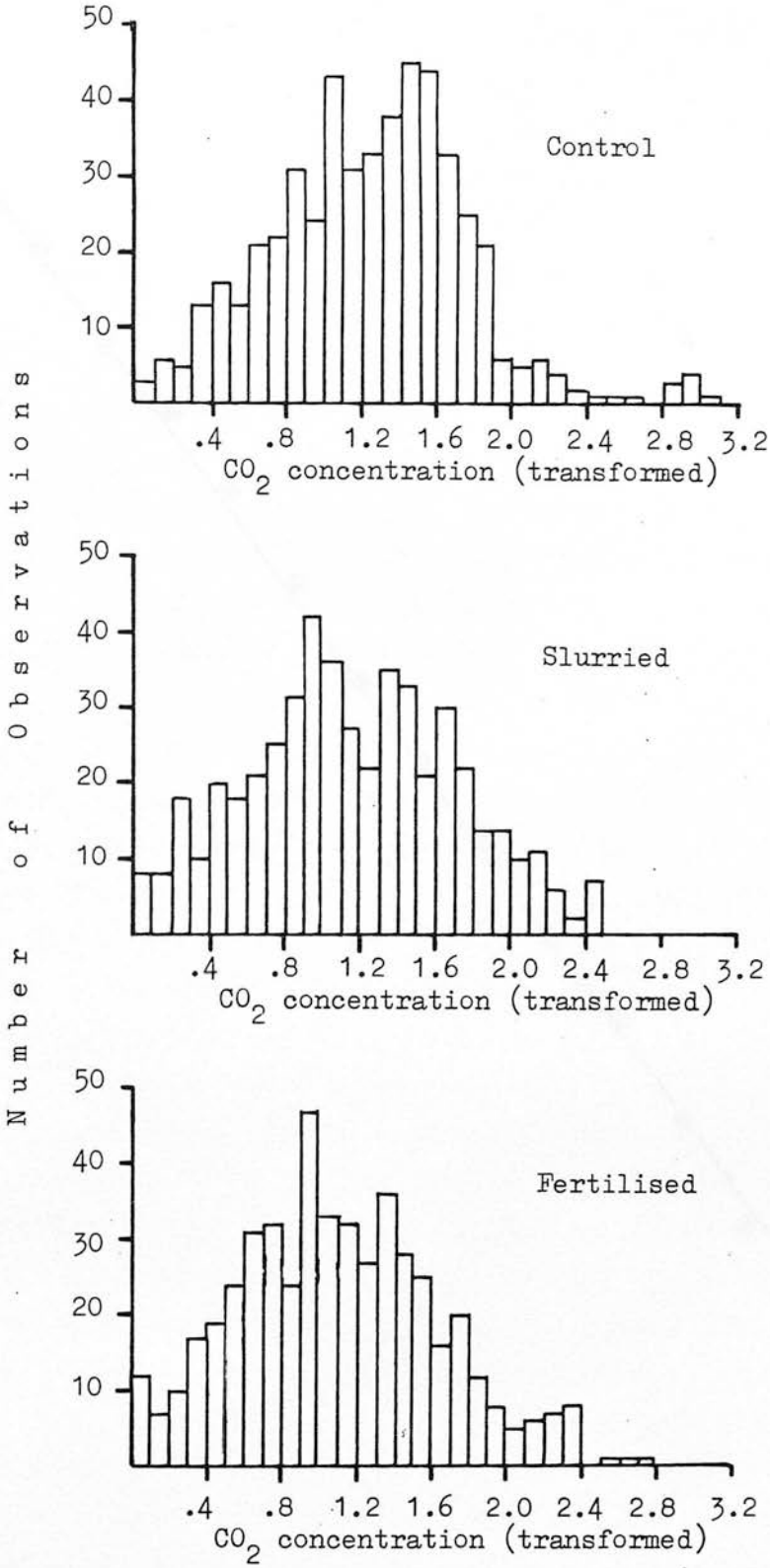


Fig. 2.8. Frequency distributions of transformed CO_2 data

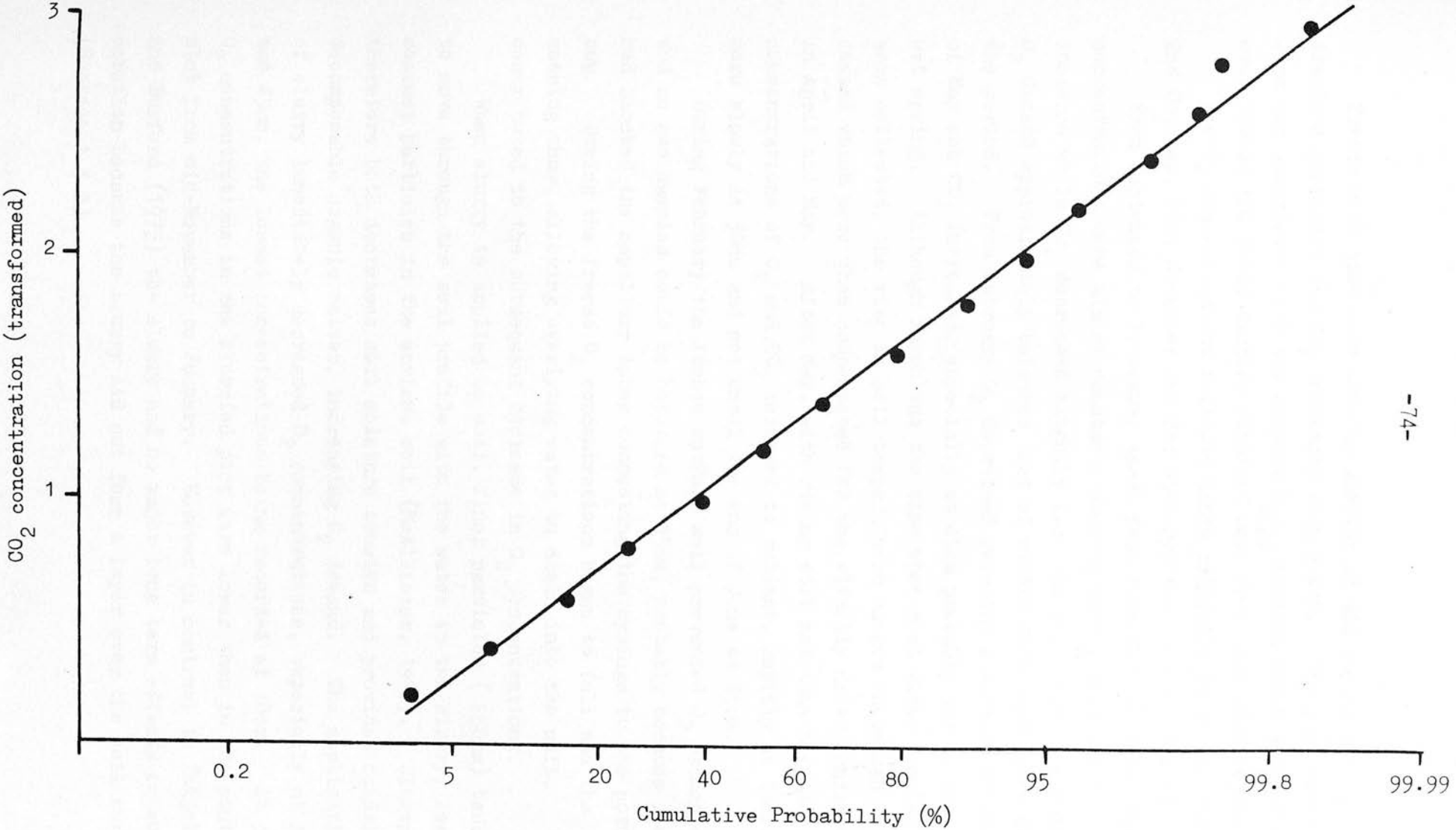


Fig. 2.9. Probability plot for combined CO_2 data (transformed)

Trends with time were usually similar at all depths but O_2 concentrations decreased and CO_2 increased with depth. The poor aeration at 45cm was associated with the compact $B_{2(g)}$ horizon which drained slowly. Over 75% of the water samples obtained were from this 45cm depth.

Low O_2 concentrations followed heavy rainfalls (e.g. 7th September, 2nd October, 20th November and 2nd January) but were shortlived.

From September to February, apart from such fluctuations, O_2 concentrations were almost constant, showing only a very small increase while CO_2 decreased slightly i.e. the influence of decreased O_2 demand approximately balanced that of wetter soil conditions over the period. From February O_2 decreased reaching a minimum at the end of May and CO_2 increased, especially at 45cm probably due to the wet spring. Although March was the time when most water samples were collected, the rise in soil temperatures caused increased O_2 demand which more than compensated for the slightly drier conditions in April and May. After May, with rising soil moisture tensions, concentrations of O_2 and CO_2 returned to ambient, rapidly at 15cm, more slowly at 30cm and not until the end of June at 45cm.

During February the frozen surface soil prevented O_2 exchange and no gas samples could be obtained at 15cm, probably because ice had blocked the capillary tubes connecting the syringe to the porous cup. During the freeze O_2 concentrations began to fall and the ensuing thaw, allowing overlying water to drain into the soil, contributed to the subsequent decrease in O_2 concentrations.

When slurry is applied to soil, finer particles (250μm) tend to move through the soil profile with the water in the slurry, leaving coarser particles in the surface soil (McAllister, 1977). Slurry therefore both decreases soil moisture tension and provides readily decomposable organic matter, increasing O_2 demand. The application of slurry immediately decreased O_2 concentrations, especially at 30 and 45cm, the lowest concentrations being recorded at 30cm. At 30cm O_2 concentrations in the slurried plot were lower than in the control plot from mid-November to January. However in contrast to Thijell and Burford (1975) the slurry had no major long term effects on soil aeration because the slurry did not form a layer over the soil surface (Section 1.3.3).

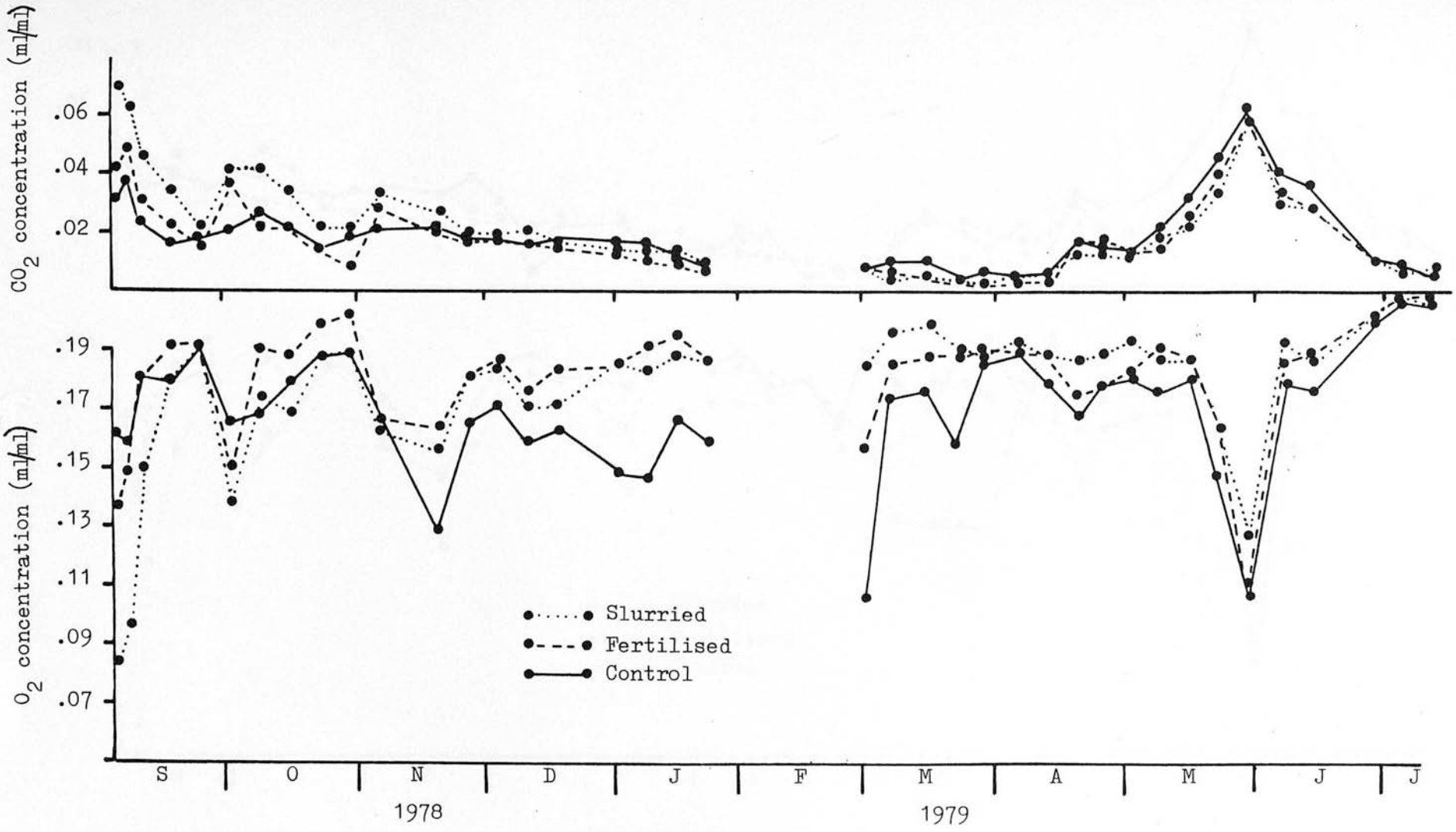


Fig. 2.10. Mean O_2 and CO_2 concentrations at the 15cm depth

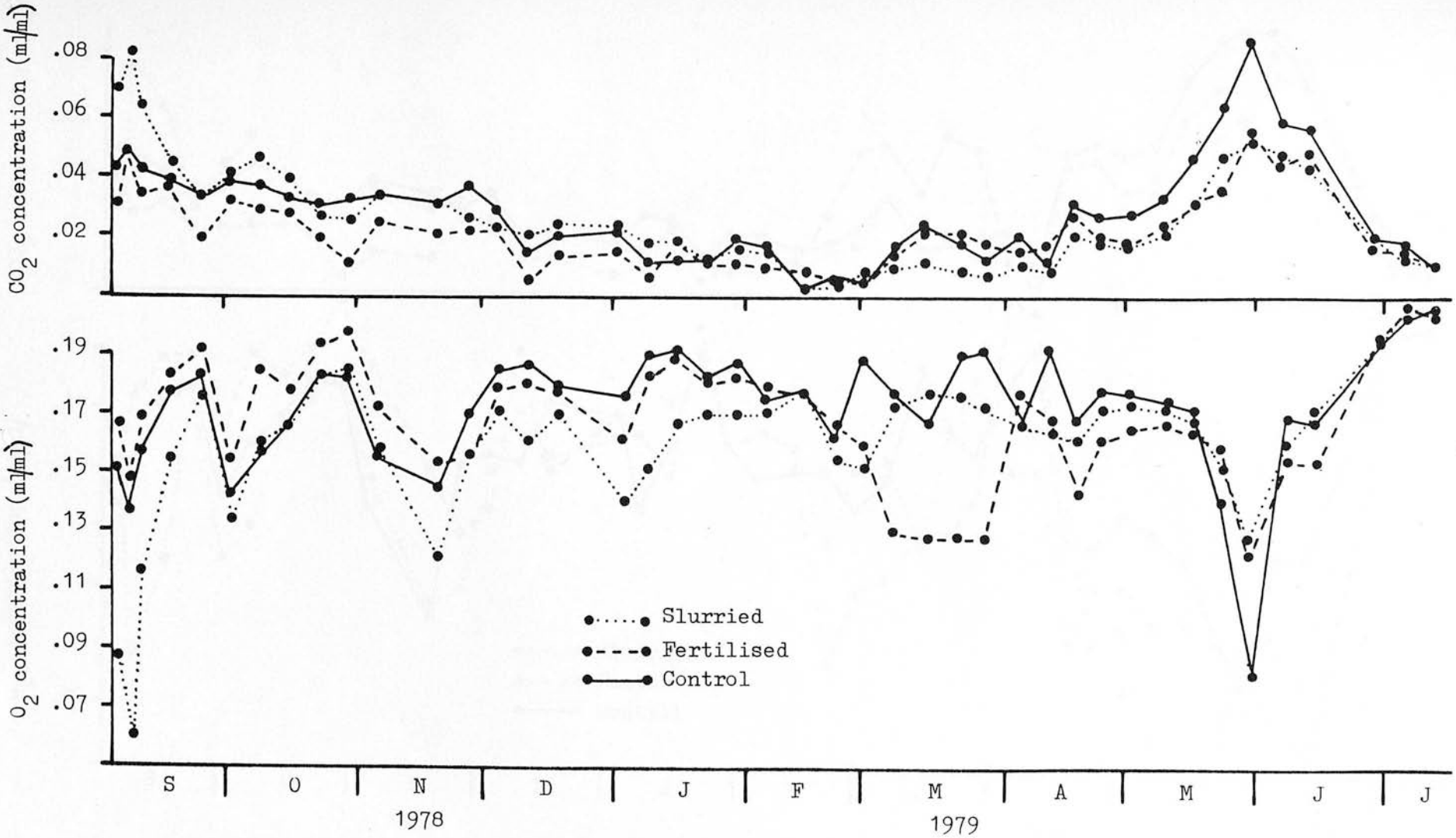


Fig. 2.11. Mean O_2 and CO_2 concentrations at the 30cm depth

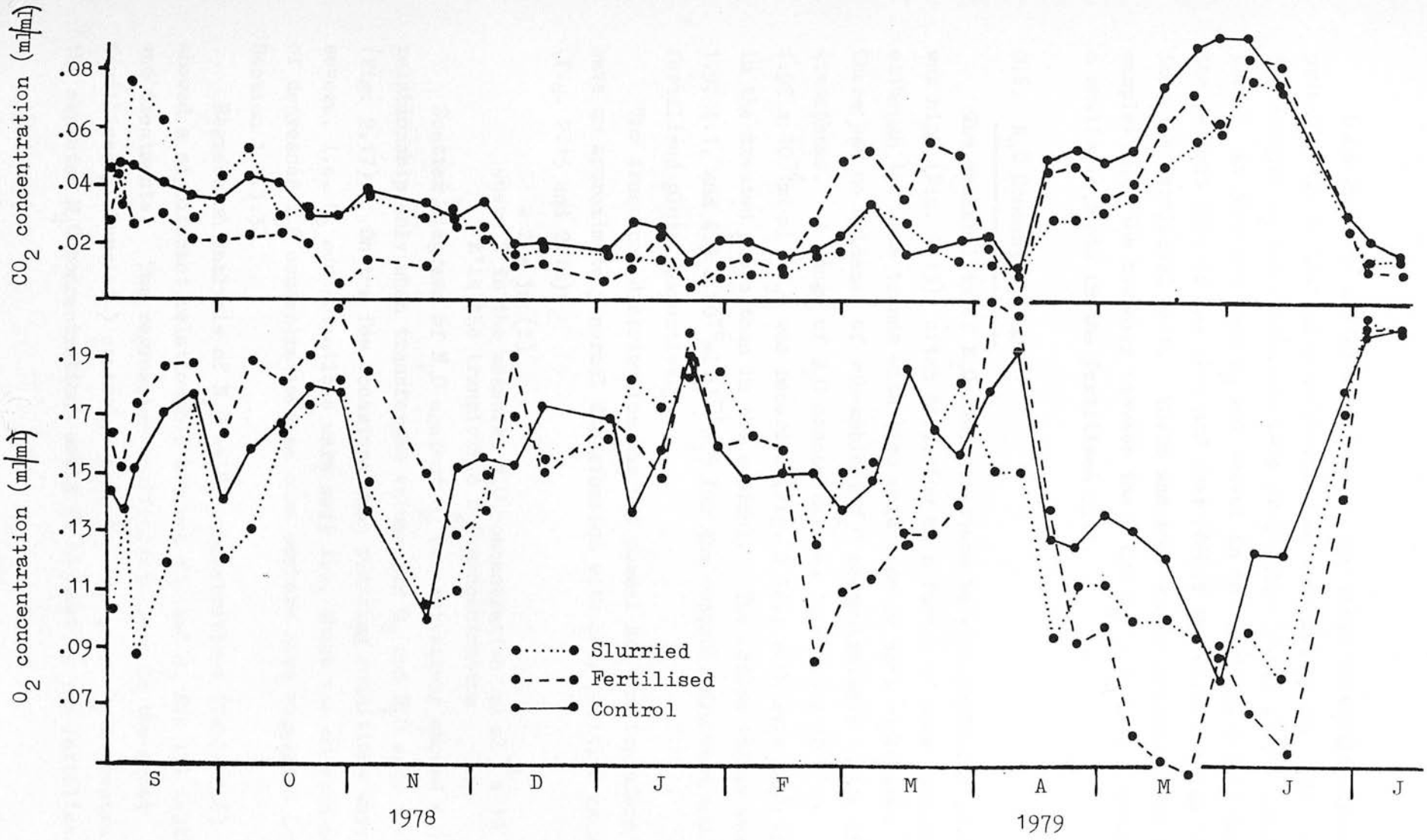


Fig. 2.12. Mean O_2 and CO_2 concentrations at the 45cm depth

Some apparent differences between the three treatments were probably due to intrinsic differences between the plots. At 15cm for example O_2 concentrations were frequently lowest in the control plot. At 30cm and 45cm O_2 was lowest in the fertilised plot during the wettest time of the year and over 60% of the water samples came from the fertilised plot. There was some visual evidence from core samples that the boundary between the S and $B_{2(g)}$ horizon occurred at a shallower depth in the fertilised plot.

2.6. N_2O Concentrations

The variability of N_2O concentrations between replicate probes was high (Fig. 2.13), often differing by a factor of more than 10, although the same trends with time were seen in each replicate. There was no evidence of sub-ambient N_2O concentrations in the soil atmosphere. A range of N_2O concentrations from 0.3×10^{-6} to 4.92×10^{-4} ml ml^{-1} N_2O was recorded (Fig. 2.14), with more high values in the treated plots than in the control. The median values were 1.9, 4.1, and 4.3×10^{-6} ml ml^{-1} N_2O for the control, slurried and fertilised plots respectively.

The frequency distribution was not normal but the transform below gave an approximately normal distribution with only positive values (Fig. 2.15 and 2.16):

$$Z' = 2 + \ln (Z) \qquad 2.3$$

where Z is the measured N_2O concentration (ml ml^{-1} x 10^{-6})
Z' is the transformed N_2O concentration

Scatter diagrams of N_2O against O_2 concentrations showed a relationship only when transformed values for O_2 and N_2O were used (Fig. 2.17). On the few occasions when reducing conditions were severe, i.e. O_2 concentrations were very low, there was no evidence of decreased N_2O concentrations as some workers have suggested (see Section 1.5.1.3).

Regression analysis of N_2O on O_2 concentrations (Table 2.6) showed a significant relationship between N_2O and O_2 for all depths and treatments. The regression coefficients were in the order fertilised > slurried > control; i.e. for any given O_2 concentration the expected N_2O concentrations would be highest in the fertilised

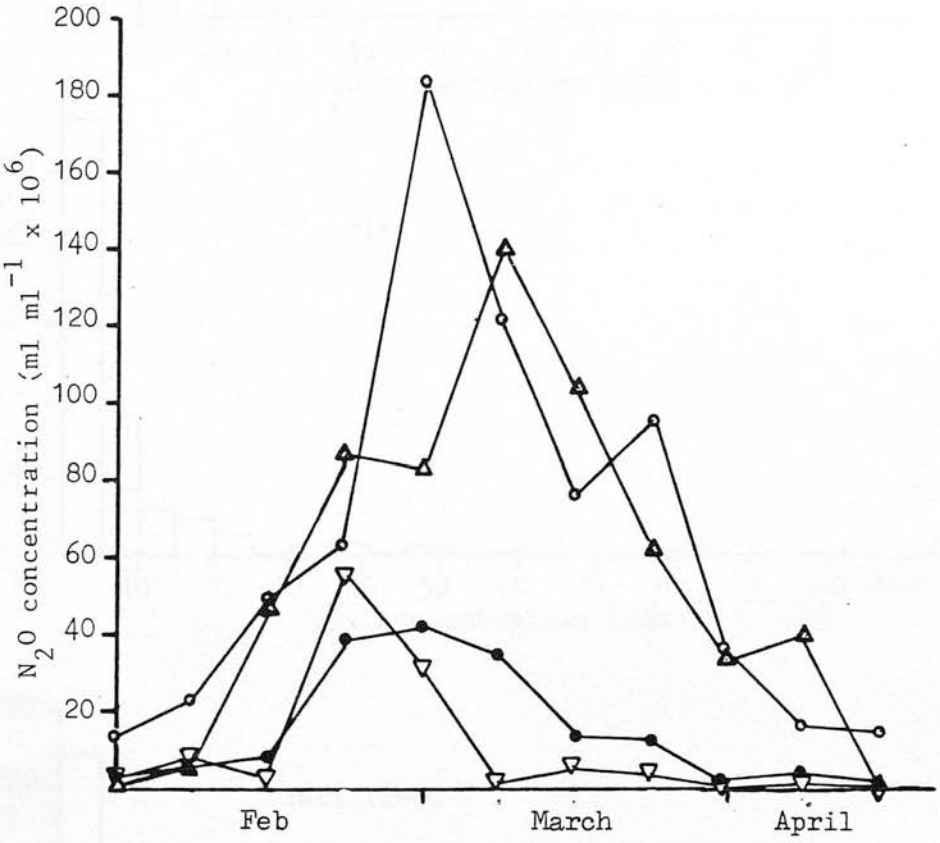


Fig. 2.13. Variability of N_2O concentrations at 4 replicate sampling points at 45cm (slurried plot)

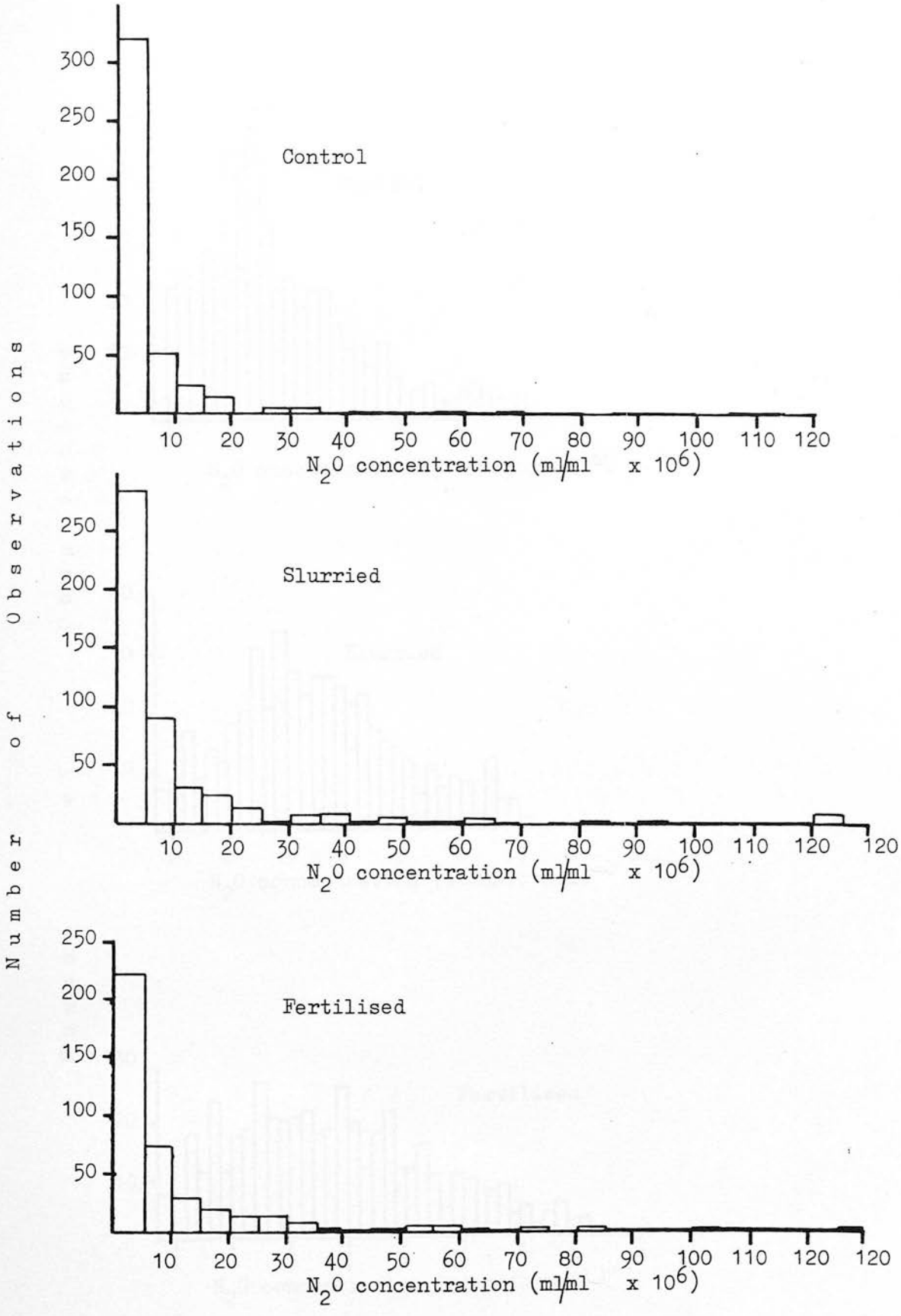


Fig. 2.14. Frequency distributions of untransformed N_2O data

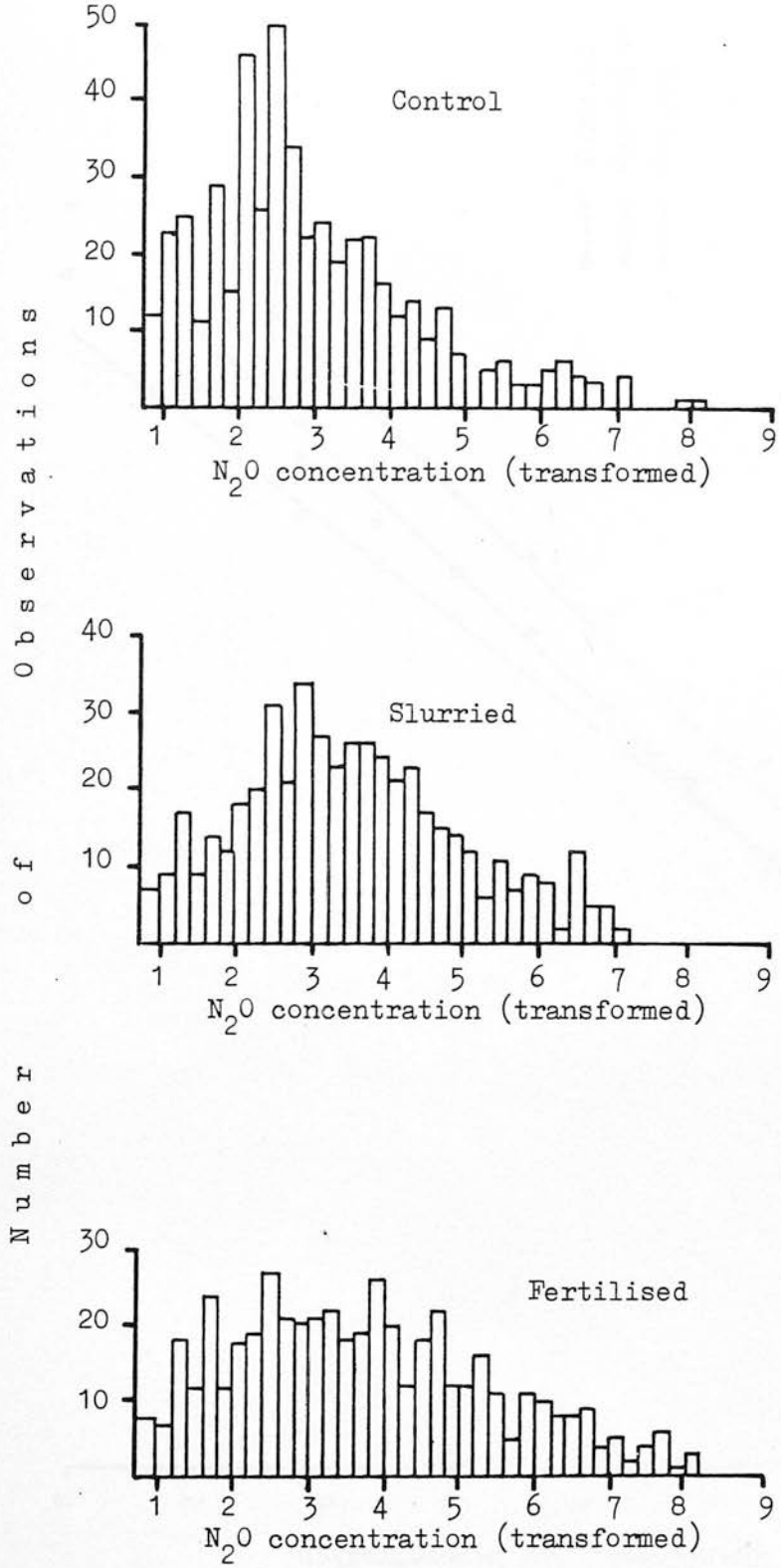


Fig. 2.15. Frequency distributions of transformed N_2O data

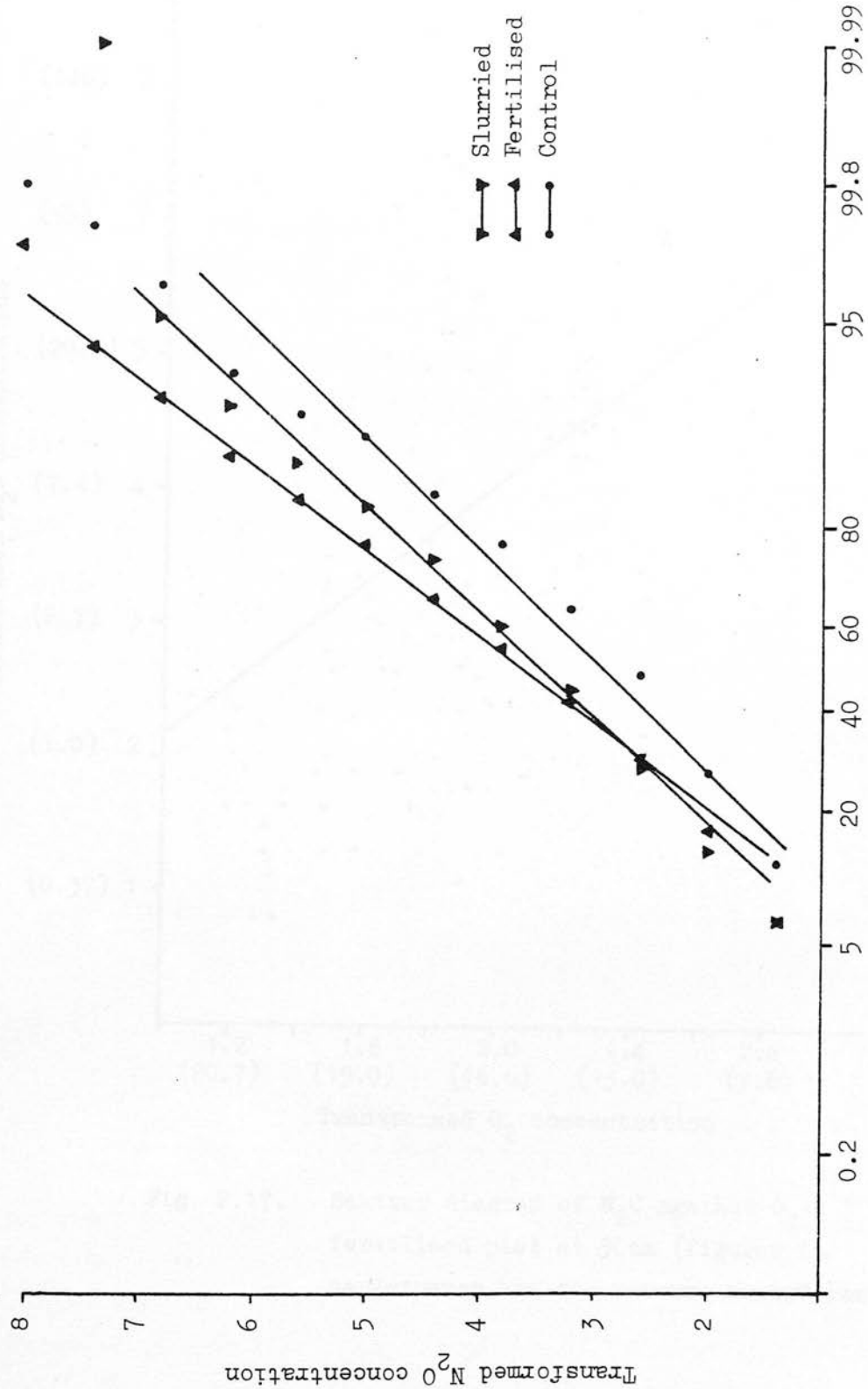


Fig. 2.16. Probability plot of N_2O data (untransformed)

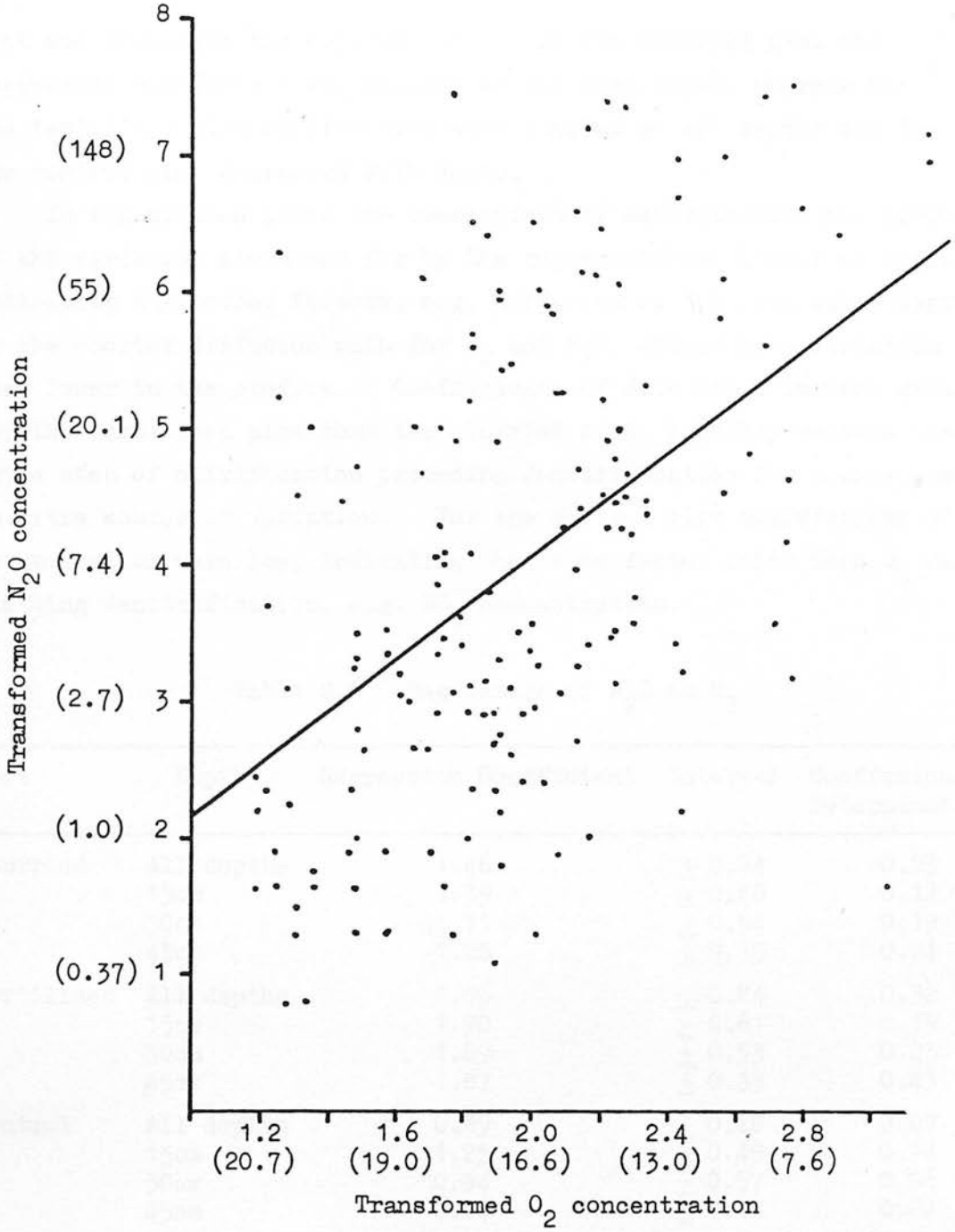


Fig. 2.17. Scatter diagram of N_2O against O_2: fertilised plot at 30cm (figures in parentheses are the reverse transforms)

plot and lowest in the control plot. In the slurried plot the regression coefficient was highest at the 30cm depth, whereas in the fertilised plot coefficients were similar at all depths and in the control plot decreased with depth.

In the treated plots the coefficient of determination (the proportion of the variation accounted for by the regression) was lowest at 15cm, indicating that other factors, e.g. diffusion of N_2O from other depths or the shorter diffusion path for O_2 and N_2O, caused more variation than lower in the profile. Coefficients of determination were greater for the fertilised plot than the slurried plot, possibly because the extra step of nitrification preceding denitrification for slurry was an extra source of variation. For the control plot coefficients of determination were low, indicating that some factor other than O_2 was limiting denitrification, e.g. NO_3^- concentration.

Table 2.6 Regression of N_2O on O_2

Plot	Depth	Regression Coefficient	Interval	Coefficient of Determination
Slurried	All depths	1.46	± 0.24	0.23
	15cm	1.39	± 0.48	0.17
	30cm	1.71	± 0.54	0.19
	45cm	1.28	± 0.35	0.24
Fertilised	All depths	1.86	± 0.24	0.32
	15cm	1.90	± 0.61	0.19
	30cm	1.89	± 0.53	0.23
	45cm	1.87	± 0.33	0.43
Control	All depths	0.89	± 0.28	0.07
	15cm	1.25	± 0.49	0.14
	30cm	0.94	± 0.57	0.06
	45cm	0.64	± 0.48	0.04

Transformed values of N_2O concentrations were used to calculate the means for depths and treatments (Figs. 2.18 - 2.20).

In the control plot the variation of N_2O concentrations with time was similar at all depths. Concentrations of N_2O were highest at 30cm, although aeration was much worse at 45cm (see Section 2.5), probably because of low NO_3^- concentrations, due to slow nitrification

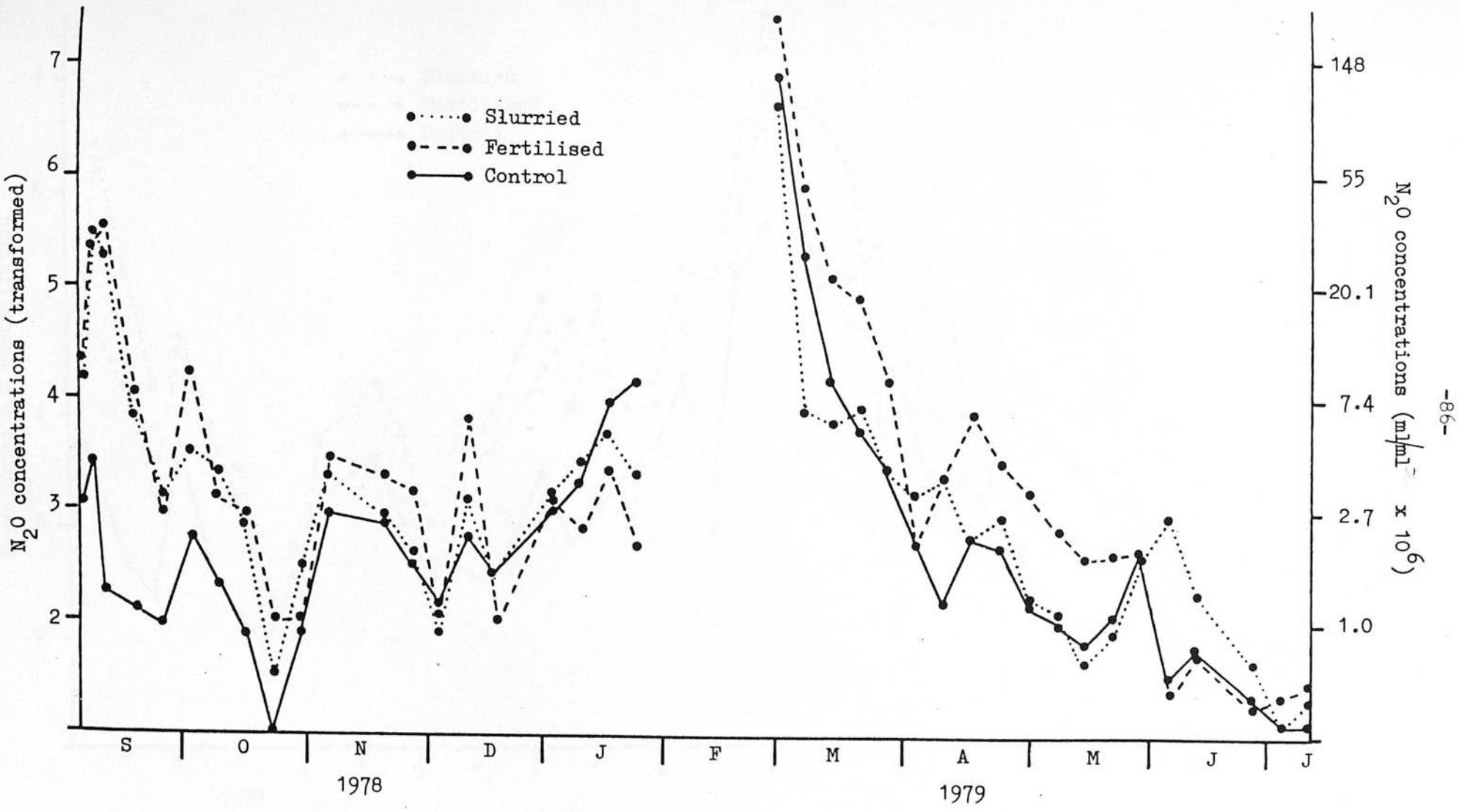


Fig. 2.18. Mean N_2O concentrations at the 15cm depth

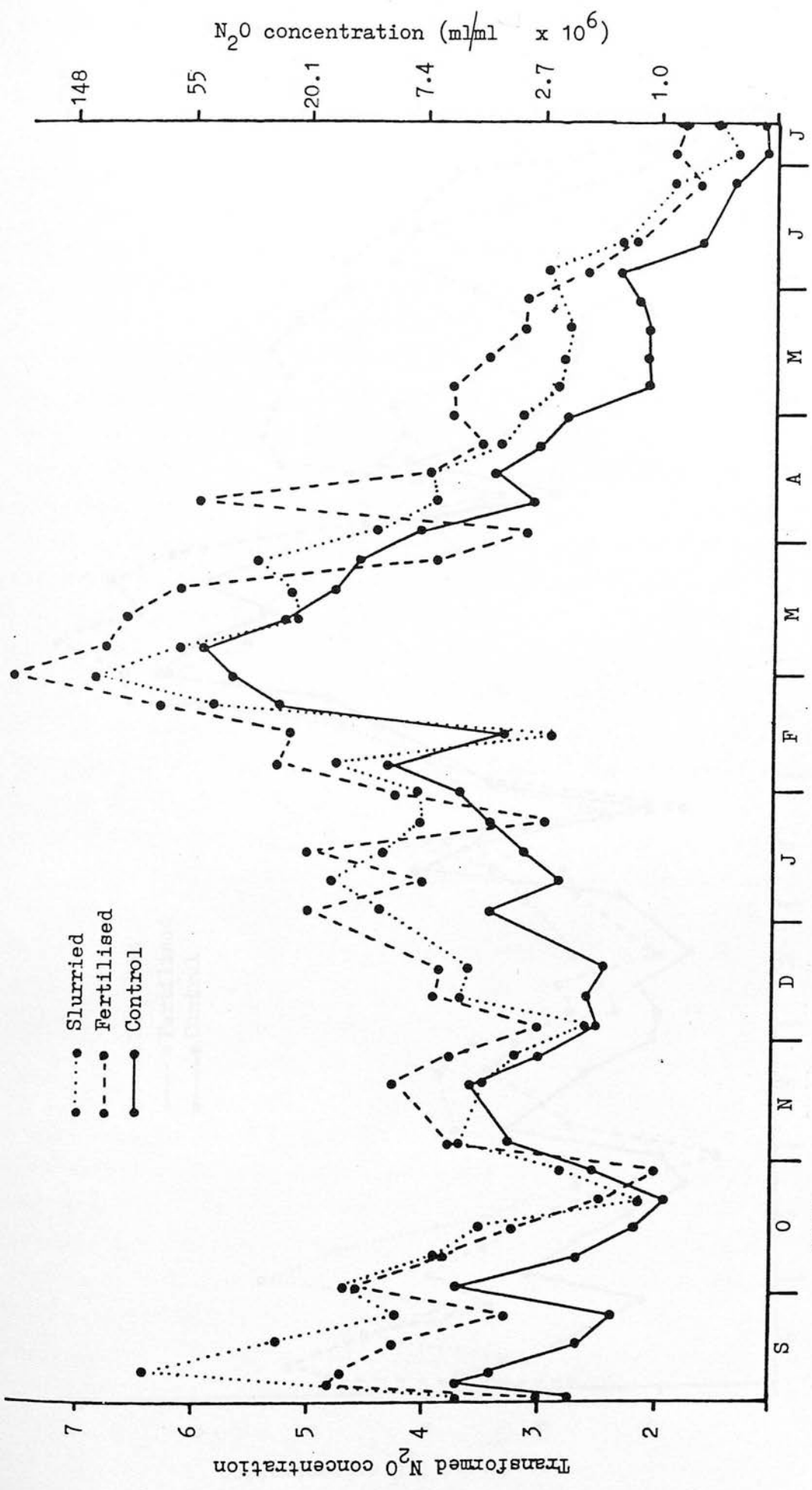


Fig. 2.19. Mean N_2O concentrations at the 30cm depth

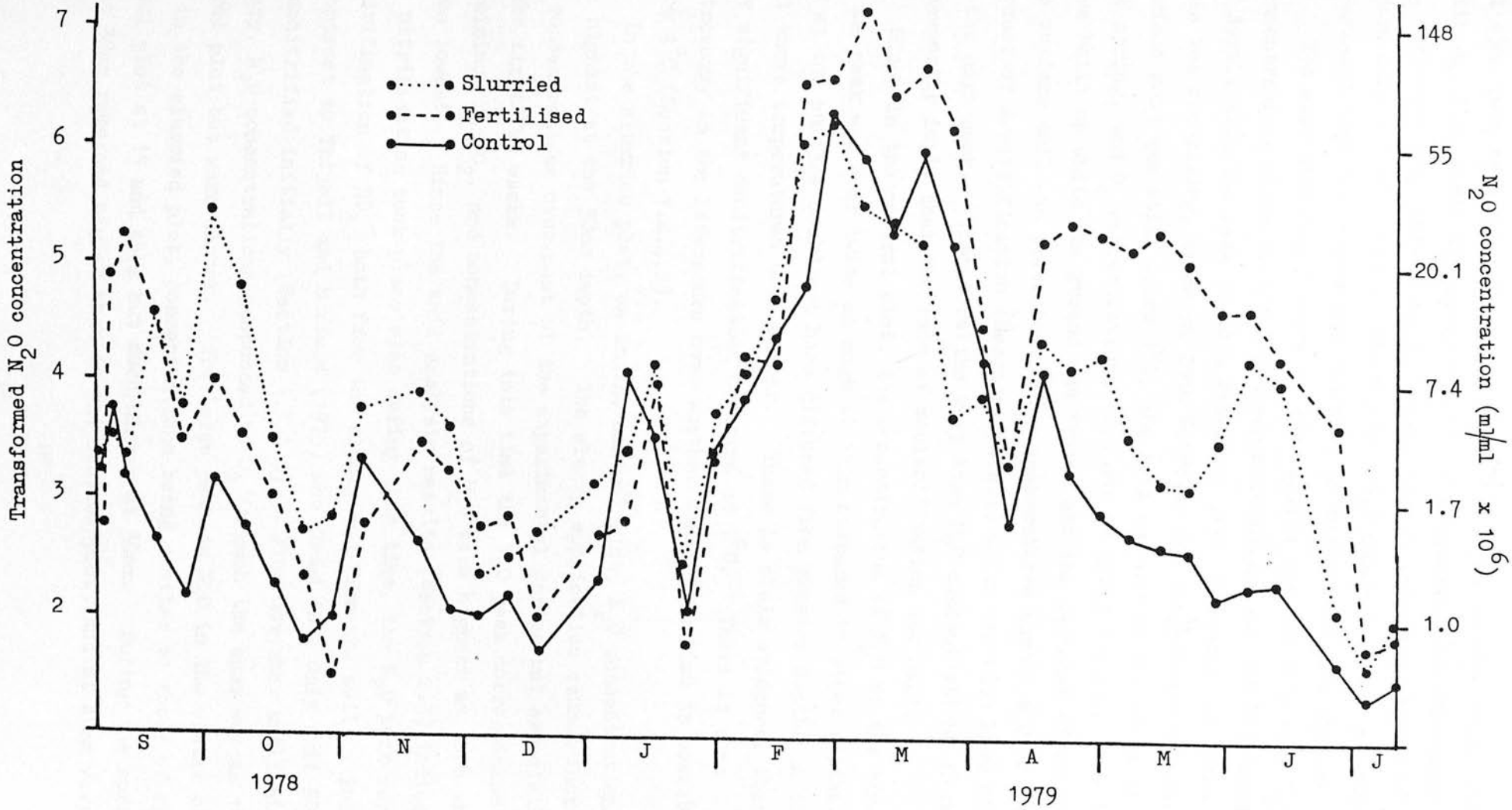


Fig. 2.20. Mean N_2O concentrations at the 45cm depth

at 45cm (see Section 2.3). There was a general inverse relationship with O_2, i.e. until the early winter N_2O concentrations increased when O_2 decreased and vice-versa, but there was no underlying trend of increasing N_2O with time. Thereafter, N_2O concentrations generally increased over the winter and returned to ambient in late spring.

The most striking feature of the results was the very high N_2O concentrations which occurred between mid-January and the beginning of April, with the peak on 28th February, just following the thaw. This was surprising, since on 28th February the temperature of the surface soil was still below 1°C, the soil was not as wet as later in the spring, and O_2 concentrations were not at their lowest. The N_2O may have built up while the ground was frozen and the exchange of gases through the surface soil was prevented. Low temperatures favour N_2O as the product of denitrification (Section 1.4.4.2) which may also have contributed to the high peak. Therefore the very high N_2O concentrations do not necessarily imply that the rate of denitrification was high.

Since in the control plot, the concentration of N_2O at the time of the peak was over twice as high at 15cm compared to other depths, N_2O at the surface could not have diffused from greater depths in the soil where temperatures were warmer. There is clear evidence, therefore, that significant denitrification occurred at 1°C. There is some controversy in the literature over whether denitrification is possible below 4°C (Section 1.4.4.2).

In the slurried plot, as in the control plot, N_2O concentrations were highest at the 30cm depth. The slurry application caused increased N_2O concentrations over most of the experimental period but especially in the first few weeks. During this time the N_2O peak corresponded to the miminum for O_2, and concentrations of N_2O were highest at 30cm where O_2 was lowest. Since the soil analysis results (Section 2.3) indicated that nitrification took place also during this time, the N_2O peak represented denitrification of NO_3^- both from the slurry and from the soil. This is in contrast to Thijell and Burford (1975) who found that only soil NO_3^- was denitrified initially (Section 1.5.1.4). From November until mid-January, N_2O concentrations responded to O_2 in much the same way as the control plot but were higher. The large peak in N_2O in the winter occurred also in the slurried plot, concentrations being similar to those of the control plot at 15 and 45cm but much higher at 30cm. During the spring N_2O at 30cm remained higher than in the control plot, and at 45cm very much

higher, reflecting the lower O_2 concentrations and high NO_3^- concentrations (Section 2.3) in the slurried plot.

If the slurry had remained on the soil surface, then nitrification and denitrification would have been limited to the surface soil, and depths to which NO_3^- had leached. The fact that increased N_2O concentrations were found at all depths indicates that some of the slurry had moved downwards, however,it is possible that denitrification occurred mostly in the top 10cm of soil where there were no soil atmosphere probes. The soil analysis results also indicated movement of the slurry through the profile (Section 2.3). Since inorganic N, about 50% of the total N in slurry according to most workers (Tunney, 1981) had largely disappeared three weeks after application (Section 2.3) the increased N_2O in the early winter, spring must have arisen from NO_3^- from the mineralisation and nitrification of some of the organic matter from the slurry remaining in the soil profile. This was likely to be a small proportion of the applied N since it is estimated that about 50% of slurry organic matter N is mineralised in the first year, and most of this occurs in the spring and summer.

The fertiliser also had an immediate effect, especially at 15cm but concentrations of N_2O were lower than in the slurried plot. After two weeks, N_2O concentrations followed the same trends in the fertilised plot as in other plots but were higher, especially at the 30cm depth. At the time of the peak on 28th February, concentrations of N_2O were over twice as high in the fertilised plot as in the control plot and N_2O remained higher until the end of the experiment, especially at 45cm. This may reflect the poorer aeration during the winter in the fertilised plot rather than the treatment, since NO_3^- concentrations and N uptake (Section 2.3) were similar in the fertilised and control plots during this time.

In spite of the large slurry application, there were lower concentrations of N_2O in the profile of the slurried plot than in the fertilised plot which received much less N as inorganic fertiliser, i.e. the nitrification step slowed down denitrification.

2.7. Statistical Analysis

For the analysis the data were divided into the 4 periods below:

(1) 15th Sept. - 16th Oct - the 6 weeks of maximum treatment effects

(2) 23rd Oct. - 15th Jan - a period of low N_2O concentrations

(3) 22nd Jan. - 19th April - the period of the peak in N_2O concentrations

(4) 25th April - 12th July - a period of declining N_2O concentrations

For each period the mean value of O_2 and N_2O was calculated for each probe using transformed values, and these means were used for an analysis of variance (Appendix 7B) and to calculate the mean values for N_2O and O_2 for each depth and treatment over each period (Fig. 2.21 and Table 2.7 and Fig. 2.22 and Table 2.8 for N_2O and O_2 respectively).

During period 1, N_2O concentrations in the control plot were significantly lower than in the other plots, but there was no significant difference between the slurried and fertilised plots and although N_2O was highest at 30cm the difference was not quite significant. Slurry significantly decreased O_2 concentrations, indicating that the high N_2O concentrations in the slurried plot could have resulted from low O_2 as well as increased NO_3^-. Also, O_2 concentrations were significantly higher in the fertilised than the control plot, i.e. the high N_2O

concentrations in the fertilised plot occurred in spite of higher O_2 concentrations. Oxygen concentrations were significantly higher at 15cm than at other depths.

In period 2 there were no significant treatment differences for O_2 or N_2O but N_2O was significantly higher at 30cm than other depths, while O_2 was significantly lower at 45cm, i.e. high N_2O concentrations at 30cm were probably due to higher NO_3^- concentrations and not to lower O_2.

In period 3, N_2O concentrations were significantly higher in the fertilised plot and at 15cm while O_2 was significantly higher at 15cm than other depths, but treatment differences were not significant. Thus the high N_2O peak in the fertilised plot was probably due to the fertiliser rather than to plot differences, and N_2O was highest at 15cm in spite of higher O_2 concentrations.

During the final period there were significant differences between treatments for N_2O but not for O_2, indicating that differences in N_2O may have been due to treatment effects. Nitrous oxide concentrations were significantly higher at 45cm than at other depths, while O_2 concentrations decreased significantly with depth.

Thus the broad trends described in Section 2.5 and 2.6 were statistically significant: i.e. O_2 decreased with depth; slurry only affected O_2 concentrations in the first few weeks; N_2O concentrations at 30cm were higher than at 15cm; initially N_2O concentrations were highest at 30cm but during the final period were highest at 45cm.

The analysis of variance using the means for each probe over the entire experimental period showed that the treatments significantly increased N_2O but that the slightly higher N_2O concentrations in the fertilised plot than the slurried plot were not significant. Overall, N_2O concentrations were significantly lower at 15cm than at other depths. Treatment differences for O_2 were not significant but O_2 decreased with depth, the difference between 15cm and 30cm being mo most marked.

The combined analysis of variance for all time periods showed that, for N_2O, changes over time were very significant. The high treatment x time interaction showed that the treatments affected N_2O

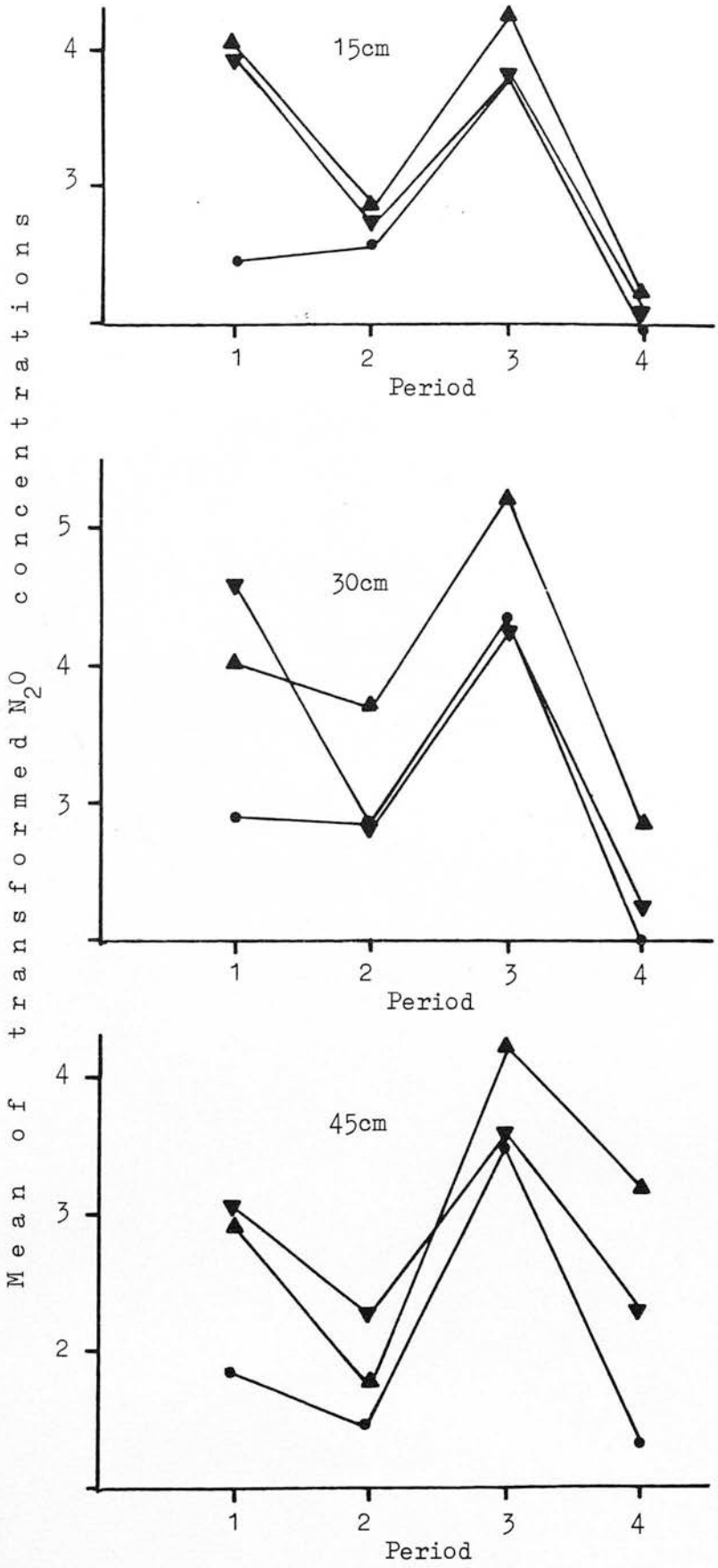


Fig. 2.21. Mean N_2O concentrations during 4 periods

▲——▲ fertilised ▼——▼ slurried ●——● control

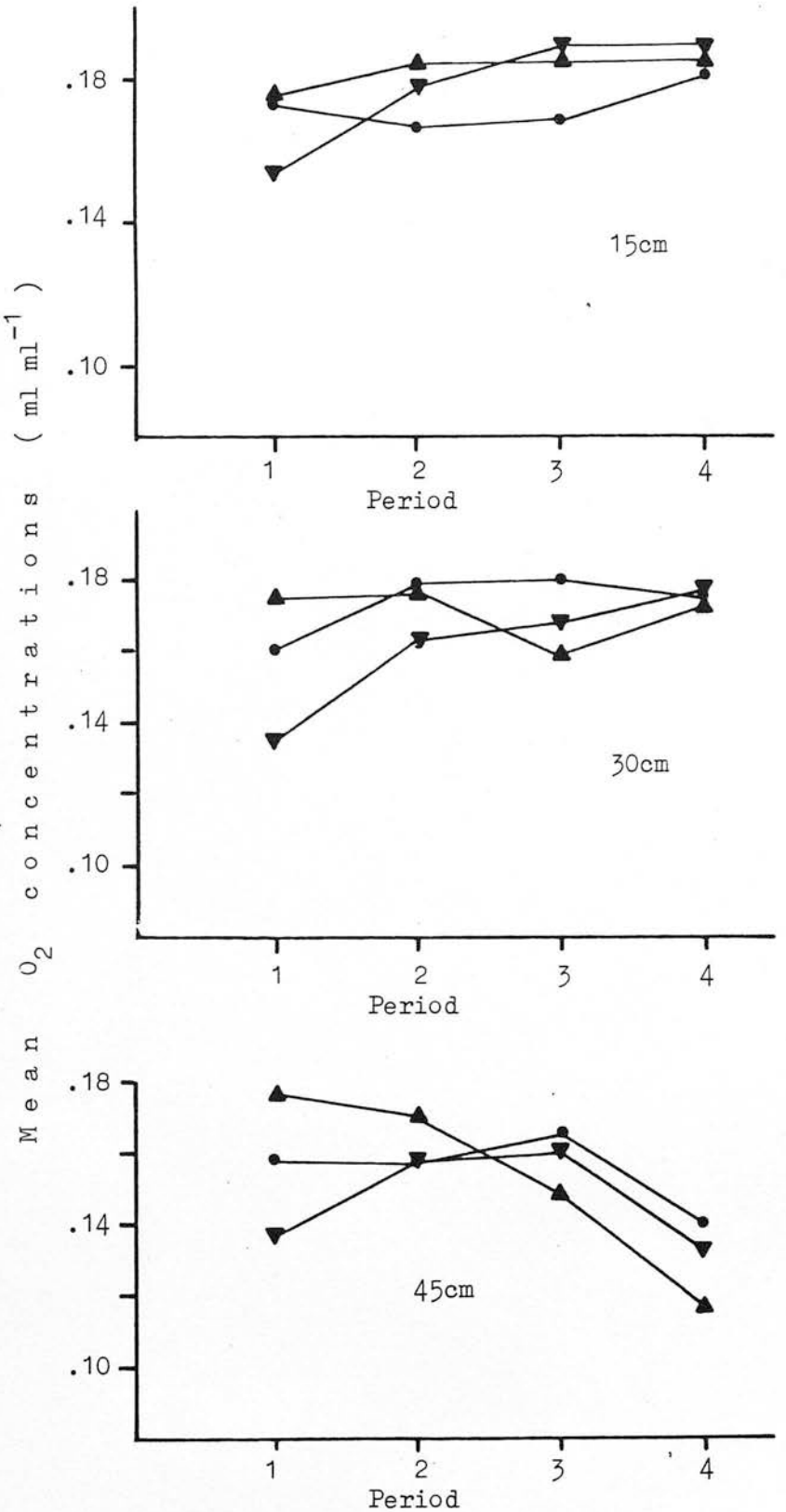


Fig. 2.22. Mean O_2 concentrations during 4 periods

▲—▲ fertilised ▼—▼ slurried ●—● control

Table 2.7 Means for N_2O during the periods analysed

	Means during period				Least significant difference at 0.05 level
	1	2	3	4	
Overall Mean	3.64 (5.16)	2.96 (2.61)	4.53 (12.55)	2.57 (1.77)	0.22
Control	2.74 (2.10)	2.65 (1.92)	4.25 (9.49)	2.04 (1.04)	0.38
Slurried	4.18 (8.85)	3.15 (3.16)	4.42 (11.25)	2.61 (1.84)	0.38
Fertilised	4.00 (7.39)	3.10 (3.00)	4.92 (18.54)	3.07 (2.92)	0.38
15cm	3.47 (4.35)	2.72 (2.05)	3.99 (7.32)	2.02 (1.02)	0.38
30cm	3.85 (6.36)	3.33 (3.78)	4.81 (16.61)	2.43 (1.54)	0.38
45cm	3.60 (4.95)	2.84 (2.32)	4.78 (16.12)	3.26 (3.53)	0.38

Table 2.8 Means for O_2 during the periods analysed

	Means during period				Least significant difference at 0.05 level
	1	2	3	4	
Overall Mean	2.05 (16.2)	1.93 (17.1)	1.93 (17.1)	1.99 (16.7)	0.08
Control	2.03 (16.4)	1.98 (16.8)	1.92 (17.2)	1.98 (16.8)	0.14
Slurried	2.25 (14.5)	1.99 (16.7)	1.89 (17.4)	1.94 (17.0)	0.14
Fertilised	1.86 (17.6)	1.83 (17.8)	1.99 (16.7)	2.04 (16.3)	0.14
15cm	1.96 (16.9)	1.83 (17.8)	1.75 (18.2)	1.70 (18.5)	0.14
30cm	2.10 (15.8)	1.90 (17.3)	1.95 (17.0)	1.87 (17.5)	0.14
45cm	2.10 (15.8)	2.06 (16.2)	2.10 (15.8)	2.40 (13.0)	0.14

N.B. Figures in brackets are the reverse transform of the means (ml ml^{-1} x 10^6 for N_2O and ml ml^{-1} x 10^2 for O_2

differently at different times. The time x depth interaction was significant because initially N_2O was similar at all depths, then highest at 30cm, and later at 45cm. For O_2 the slight increase in O_2 concentrations during the winter was significant. The depth x time interaction was significant because differences between depths increased over the winter and spring. The treatment x time interaction was significant because slurry only affected O_2 concentrations during Period 1 and because in the fertilised plot, initially O_2 concentrations were highest, while in Periods 3 and 4 they were lowest.

In the analysis above, differences ascribed to treatments could in fact be due to intrinsic plot differences. Although O_2 concentrations were not always significantly different in the three plots, there was some evidence for a trend of poorer aeration from the control to the fertilised plot in the winter. A randomised block design, with each plot sub-divided into three and forming a block, would be more suitable.

Analysis of variance has shown that replication was not always sufficient for treatment and depth differences to be significant. Increased replication would therefore improve the design. If each plot in the randomised block experiment contained two replicates at each depth, the replication would increase from four to six. Without increasing the size of the plots, replication could not be increased further.

2.8 Conclusions

The methods of sampling the soil atmosphere were satisfactory except when the ground was frozen. For the range of concentrations occurring in the samples the methods of analysis were adequate.

Logarithmic transformations of the data were required to normalise the frequency distributions for all gases, although previous workers found this necessary only for N_2O (see Section 1.5.1.1.) Replicate N_2O concentrations frequently differed by more than a factor of 10, agreeing with previous work (see Section 1.5.1.1). In spite of high random variation, treatment (plot) and depth differences were often statistically significant. However increased replication would be

desirable. Concentrations of O_2 were low and approximately constant during the autumn and winter until the end of February. Following the thaw, concentrations decreased, reaching a minimum at the end of May, later than would be usual because of the wet spring.

There was no evidence in the data for the soil acting as a sink for N_2O and N_2O concentrations were within the range of previously recorded values (see Section 1.5.1.2).

The three plots were not identical, there being a trend from the control to the fertilised plot of lower O_2 concentrations during the winter. A randomised block design, with blocks replacing the original plots, would enable this factor to be separated from treatment effects.

Nitrous oxide concentrations were high for six weeks following the application of slurry and inorganic fertiliser and were highest in all plots during the period when the ground was frozen and in the weeks following the thaw, i.e. in the winter and early spring. The data provides evidence of denitrification at temperatures as low as 1°C. Lowest N_2O concentrations were found in the summer. Apart from Dowdell and Smith (1974) who found high N_2O concentrations in the summer in a heavy wet soil, most other workers in Britain have also reported highest N_2O concentrations over the winter months (see Section 1.5.1.5).

Previous work has established a general inverse relationship between N_2O and O_2 concentrations which is often obscured by the dependence of N_2O concentrations on other factors (see Section 1.5.1.3). The regression coefficient for the regression of N_2O on O_2 concentrations for the data presented here was significantly different from zero for all treatments and depths and accounted for about 20% of the variation in N_2O in the treated plots but only about 10% in the control plot.

Inorganic fertiliser and slurry increased N_2O concentrations during most of the period at all depths, and especially at 30 and 45cm. However it was not possible to separate effects due to intrinsic plot differences from those due to treatments.

A large slurry application affected O_2 concentrations for only about four weeks. In spite of the larger application of N to the

slurried plot, N_2O concentrations were generally lower than in the fertilised plot. The pattern of O_2 and N_2O in the slurried plot differed from that reported in a previous study (Thijell and Burford, 1975) because the slurry did not form a layer over the soil.

3. RANDOMISED BLOCK FIELD EXPERIMENT

The aim of the field experiment, which was carried out from the summer of 1979 to the summer of 1980, was to compare the effects of adding fertiliser and slurry at different times of the year on soil N_2O concentrations at 3 depths. The relationship between N_2O and O_2 concentrations throughout the year was also investigated. Available N in the soil profile and N uptake by the herbage were also measured at various times. A qualitative comparison was made between data for the two field experiments to see whether trends noted in each experiment were general or particular to that season.

3.1. Field Site

The randomised block design allowed trends within the experimental area to be separated from treatment differences (see Section 2.7). The three plots described in Section 2.1 were each sub divided into 3 plots of 3m x 8m and treatments were assigned at random within each block (Fig. 3.1 and Plate 3.1). No treatments were applied to the guard rows, 0.5m wide, between the plots within each block. Two gas sampling probes were installed at each of 3 depths in each plot, at least 0.5m from the edge of the plot, a sufficient distance to ensure that the treatment of neighbouring plots had no effect on gas samples.

3.2. Methods

Applications of slurry (for analysis see Table 3.1) and inorganic fertiliser (100kg ha^{-1} of N as calcium nitrate) were made on 16th July, 20th August, 29th October and 15th April to the three slurried and fertilised plots respectively. Applications were made only when the major effects of the previous application on O_2 or N_2O concentrations had disappeared and for this reason no application of slurry could be made in the winter of 79/80.

The grass was cut on 16th July (before the application), 9th August, 31st August, 1st October, 29th October (before the application), 21st May and 19th June. Total dry matter per unit area was determined for each plot, and N, P, and K analysis carried out (see Appendix 5). Two soil cores per plot were removed on 9th August, 1st October, 10th January, 15th April, and 22nd July, and NO_3^- concentrations were determined for all cores, and exchangeable NH_4^+ for cores from the

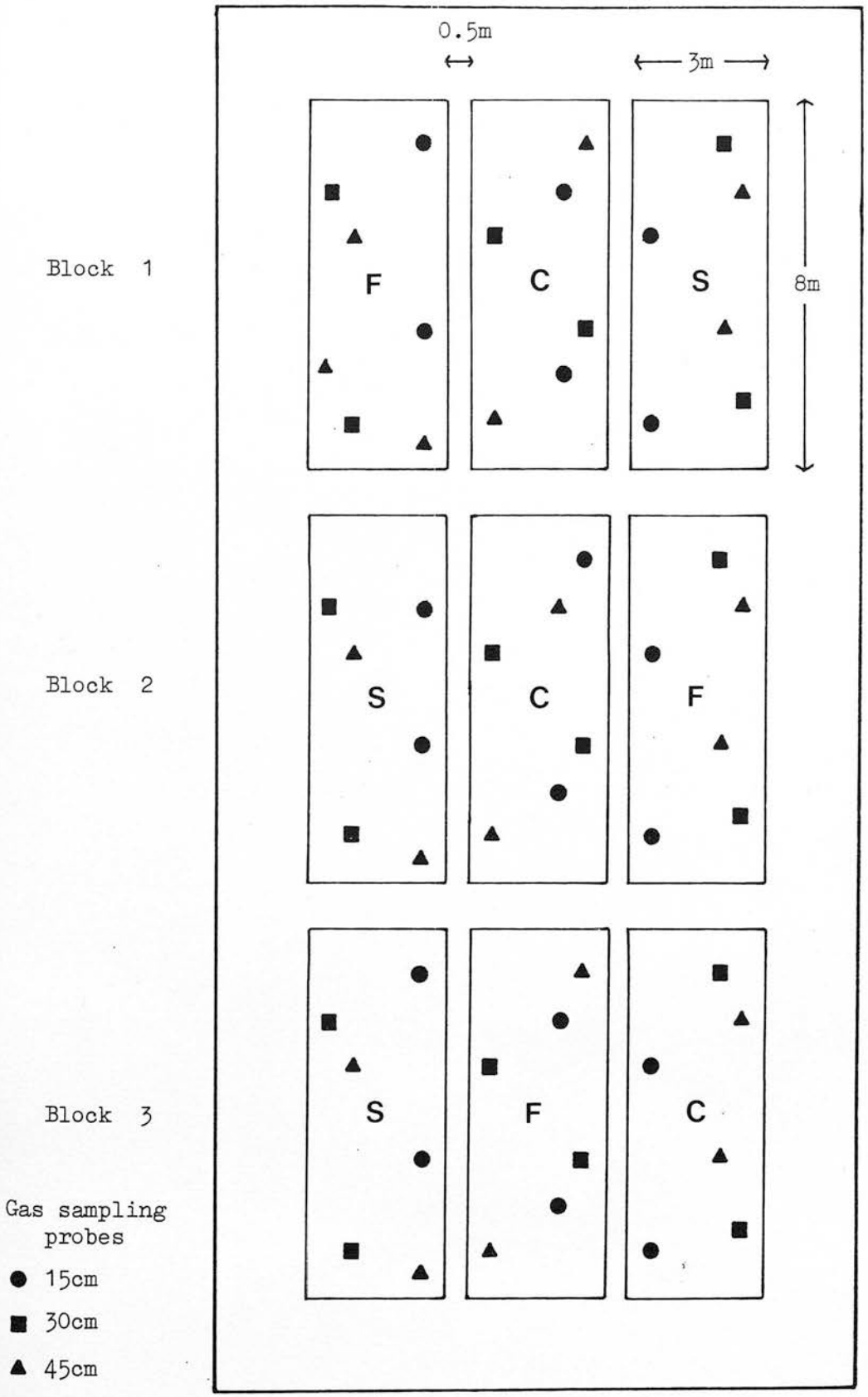


Fig. 3.1. Layout of randomised block field experiment

Plate 3.1. Randomised block field experiment

Table 3.1 Slurry applied to plots

Date of Application	Slurry analysis (%)[a] N	P	K	Volume Applied (lm^{-2})	N applied kg ha^{-1}
16. 7.79	0.110	0.024	0.170	5.4	59.4[b]
20. 8.79	0.093	0.022	0.170	8.0	74.4
29.10.79	0.075	0.021	0.100	9.5	71.3
15. 4.80	0.075	0.011	0.070	14.2	93.7

(a) See Appendix 5 (b) less applied than intended because preliminary analysis differed from analysis of slurry at time of application

control and slurried plots (except for cores of July 22nd) (see Appendix 6. Method 1 was used for NH_4^+).

Cores were taken at least 0.5m from the position of any previous core (to avoid any effects on aeration which might affect NH_4^+ and NO_3^- concentrations), at least 0.5m from the edge of the plot (to avoid unrepresentative samples), and at least 0.5m from any gas sampling probe so that gas samples would not be affected. This restricted the total number of cores which could be taken from the plots to two on each sampling occasion.

Gas or water samples were taken weekly or more frequently following an application of slurry and fertiliser (see Appendices 1-3).

3.3. Herbage Analysis

The results of the herbage analysis (Tables 3.2 and 3.3, and Fig. 3.2) showed significant differences between the three treatments, with the yield, and often N content, on the fertilised and slurried plots higher than those of the control plots. There was some evidence in the data for a reduced yield in Block 1 (not quite significant at the 0.05 level).

The overall percentage recovery of N in the herbage (the difference between the mean N uptake of the treated and control plots as a percentage of applied N) was approximately 20 and 35% for the slurried and fertilised plots respectively. This is slightly higher than the usually quoted figures for slurry of 15% (Kiely, 1981; Tunney, 1981) and indicates low N losses by leaching, volatilisation of NH_3 and denitrification during the period of study.

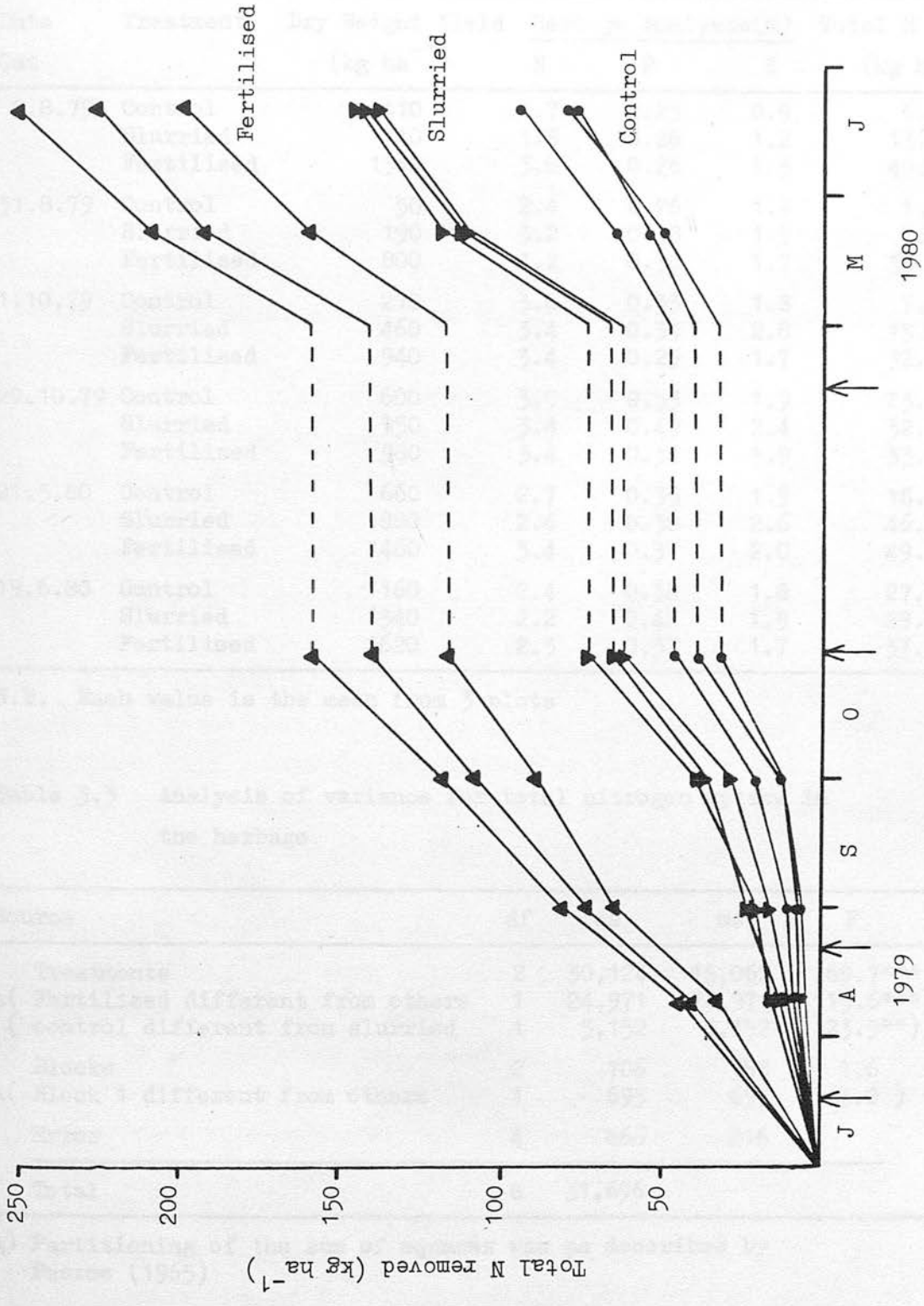


Fig. 3.2. Nitrogen removed in herbage (cumulative)
(arrows indicate fertiliser and slurry applications)

Table 3.2 Herbage Analysis

Date Cut	Treatment	Dry Weight Yield (kg ha^{-1})	Herbage Analysis(%) N	P	K	Total N Removed (kg ha^{-1})
9.8.79	Control	410	1.7	0.25	0.9	6.9
	Slurried	740	1.8	0.26	1.2	13.4
	Fertilised	1340	3.0	0.26	1.5	40.0
31.8.79	Control	50	2.4	0.26	1.3	1.1
	Slurried	190	3.2	0.38	1.9	6.2
	Fertilised	800	4.2	0.30	1.7	33.6
1.10.79	Control	210	3.6	0.33	1.8	7.9
	Slurried	460	3.4	0.34	2.8	15.5
	Fertilised	940	3.4	0.25	1.7	32.1
29.10.79	Control	600	3.9	0.53	1.9	23.4
	Slurried	950	3.4	0.49	2.4	32.7
	Fertilised	980	3.4	0.34	1.9	33.4
21.5.80	Control	660	2.7	0.35	1.9	18.1
	Slurried	1880	2.6	0.38	2.6	46.2
	Fertilised	1460	3.4	0.37	2.0	49.1
19.6.80	Control	1160	2.4	0.38	1.8	27.1
	Slurried	1340	2.2	0.42	1.5	29.2
	Fertilised	1620	2.3	0.37	1.7	37.5

N.B. Each value is the mean from 3 plots

Table 3.3 Analysis of variance for total nitrogen uptake in the herbage

Source	df	ss	ms	F
Treatments	2	30,124	15,062	69.7***
a(Fertilised different from others	1	24,971	24,971	115.6***)
(control different from slurried	1	5,152	5,152	23.9**)
Blocks	2	706	353	1.6
a(Block 1 different from others	1	695	695	3.2)
Error	4	866	216	
Total	8	31,696		

a) Partitioning of the sum of squares was as described by Pearce (1965)

There was no evidence that the fertilised plots became deficient in P or K.

3.4. Soil Analysis

On no occasion did slurry significantly increase NH_4^+ concentrations even in the surface soil, indicating rapid nitrification of NH_4^+ from the slurry or large losses of NH_3 by volatilisation. It is known that almost all the inorganic N fraction of slurry (particularly cow slurry) can be lost when slurry is applied to warm dry soil (Kiely, 1981). Thus much of the NH_4^+ in the first two applications may have been volatilised.

There was never any evidence of accumulation of NO_3^- in the slurried plots, even in the surface soil, i.e. mineralisation and nitrification of slurry remaining in the soil did not cause any significant increase in soil inorganic N. However since roughly 50% of the organic N in slurry is mineralised over the first year, about 50 kg N ha^{-1} would have been released from the applied slurry by the summer of 1980.

The variability of NO_3^- concentrations and small treatment differences (Table 3.4) meant that two cores were usually insufficient to show significant differences in the analysis of variance (Table 3.5).

For the soil samples taken on August 9th, $3\frac{1}{2}$ weeks after the first application, differences between blocks, treatments and depths were not significant, although NO_3^- was highest in the fertilised plots and lowest in Block 1. Nitrate decreased with depth, mean concentrations at 30-40cm (0.86µg g^{-1}) being only one third of those at 0-10cm (2.56µg g^{-1}).

Nitrate concentrations were higher in all plots, in samples of 1st October 2 weeks after the second application. Analysis of variance showed that the fertilised plots had significantly higher concentrations, but that there were no significant differences between control and slurried plots, and that NO_3^- was significantly lower in Block 1. Again NO_3^- decreased with depth, the average at 0-10cm being almost 3 times that at 30-40cm.

Concentrations of NO_3^- in the soil samples of 10th January, at a time when there was no N uptake by grass, were over twice as high as those of October but block and treatment differences were not significant

Table 3.4 Ammonium and nitrate concentrations in soil samples

Plot	Depth (cm)	Inorganic N in samples ($\mu g\ N\ g^{-1}$)								
		9th Aug		1st Oct		10th Jan		15th April		22nd July
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NO_3^-
Control	0-10	0.8	2.4	0.5	4.1	1.6	7.5	0.6	2.1	2.3
	10-20	0.4	1.5	0.2	3.1	0.7	7.8	0.9	2.3	2.2
	20-30	0.2	2.9	0.4	2.8	1.0	6.2	0.7	1.4	1.5
	30-40	0.3	0.8	0.5	1.3	0.3	1.7	0.7	0.7	1.6
Slurried	0-10	0.5	0.8	0.4	3.5	2.8	11.1	0.7	1.9	3.7
	10-20	0.3	1.7	0.2	2.7	0.8	9.6	0.9	2.5	3.2
	20-30	0.5	1.3	0.1	1.9	0.3	5.9	0.9	1.6	2.8
	30-40	0.3	0.6	0.6	1.4	0.9	3.2	0.5	0.8	1.0
Fertilised	0-10	-	4.5	-	4.8	-	8.1	-	2.7	3.4
	10-20	-	2.7	-	3.7	-	6.7	-	2.9	3.2
	20-30	-	1.5	-	3.3	-	6.1	-	2.3	1.6
	30-40	-	0.8	-	1.7	-	5.6	-	1.8	0.9

NB Each value is the mean of samples from 6 cores.

although NO_3^- was lowest in the control plot. Again the decrease in concentration with depth was significant.

By April 15th, just before the spring application, NO_3^- concentrations had fallen and were similar to those of August. Block and treatment differences were not significant, although concentrations were highest in the fertilised plots, and lowest in Block 1.

At the end of the experiment NO_3^- concentrations were still low,

Table 3.5 Analysis of variance of NO_3^- concentrations

Date	Source	df	ss	ms	F
9.8.79	Treatments	2	10.66	5.33	2.25
	Blocks	2	13.67	6.83	2.88
	Depths	3	13.47	4.49	1.89
	Error	26	61.57	2.37	
	Total	33	99.37		
1.10.79	Treatments	2	7.05	3.52	5.03*
	(fertilised different from others	1	5.70	5.70	8.14**)
	(control different from slurried	1	1.35	1.35	1.93)
	Blocks	2	8.19	4.09	5.84
	(Block 1 different from others	1	7.99	7.99	11.41**)
	Depths	3	30.83	10.28	14.69***
	Error	27	18.84	0.70	
	Total	34	64.91		
10.1.80	Treatments	2	16.4	8.2	0.69
	Blocks	2	6.9	3.5	0.29
	Depths	3	157.8	52.6	4.38*
	Error	28	334.8	12.0	
	Total	35	515.9		
15.4.80	Treatments	2	4.75	2.37	0.61
	Blocks	2	12.58	6.29	1.61
	Depths	3	11.11	3.70	0.95
	Error	28	109.55	3.91	
	Total	35	137.99		
22.7.80	Treatments	2	4.97	2.48	3.02
	(control different from others	1	4.01	4.01	4.89*)
	Blocks	2	13.26	6.63	8.09**
	(Block 3 different from others	1	11.01	11.01	13.43**)
	(Block 1 different from Block 2	1	2.24	2.24	2.74
	Depths	3	35.23	8.41	10.26***
	Error	28	23.05	0.82	
	Total	35	66.51		

Notes: Values for missing data were fitted by setting the residual error to zero and total degrees of freedom were reduced by 1 for every missing value.
Brackets indicate partioning of the s.s., as described by Pearce (1965).

and were significantly lower in the control plot than others, and highest in Block 3, and again decreased with depth.

Apart from samples of 10th January, NO_3^- was always lowest in Block 1 (only significantly on 1st October). More waterlogged conditions might have decreased mineralisation and increased denitrification.

Although NO_3^- concentrations from the '79/'80 data are not strictly comparable with those of '78/'79, there is evidence of lower NO_3^- concentrations in the second year.

3.5. Temperatures and Rainfall

Temperature and rainfall data (from the Bush Estate recording station) are given in Fig. 3.3.

Although the rainfall from July 1979 to June 1980 (841mm) was very similar to that of the previous year (824mm), the distribution differed markedly, the summer and spring being drier and a greater proportion of rainfall falling from October to December.

The variation of temperature with time was similar to that of the previous year (c.f. Fig. 2.3) but lowest temperatures were recorded from January to mid-February, 4 weeks earlier than in 1978, and temperatures at 15cm fell below $0^{\circ}C$ for only 6 days (not consecutive) during January.

3.6. Soil O_2 and CO_2 Concentrations

The modal O_2 concentrations for the three treatments (Fig. 3.4) were the same (0.200 - 0.205ml ml^{-1}) indicating that in general terms, O_2 concentrations were similar under the three treatments, but the median value was slightly lower for the slurried plots (0.193ml ml^{-1}) than for the control and fertilised plots (0.198ml ml^{-1}). Thus slurry had no major effects on O_2 but did increase the frequency of low concentrations. Modal concentrations for the '79/'80 experiment were much closer to ambient O_2 concentrations (0.21ml ml^{-1}) than in the preliminary experiment and there were fewer low O_2 concentrations: e.g. 13% of observations below 0.15ml ml^{-1} compared with 28% in the preliminary experiment.

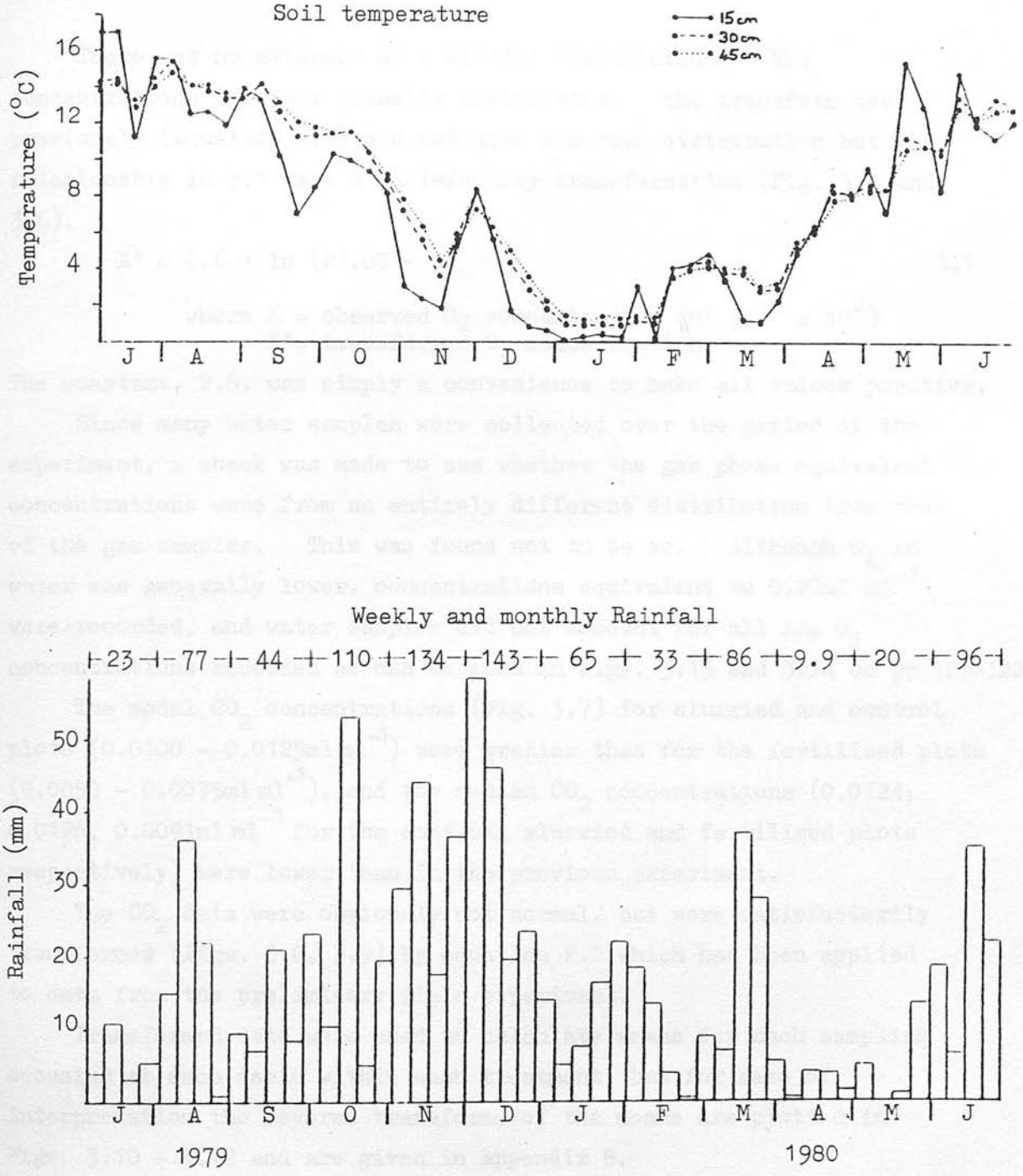


Fig. 3.3. Soil temperature at the 15, 30 and 45cm depth and weekly and monthly rainfall from July 1979 - June 1980

There was no evidence of a bimodal distribution. The concentrations were not normally distributed. The transform used previously (Equation 2.1) did not give a normal distribution but the relationship in 3.1 gave a satisfactory transformation (Fig. 3.5 and 3.6).

$$X' = 2.6 + \ln (21.09 - X) \qquad 3.1$$

where X = observed O_2 concentration (ml ml^{-1} x 10^2)
X' = transformed O_2 concentration

The constant, 2.6, was simply a convenience to make all values positive.

Since many water samples were collected over the period of the experiment, a check was made to see whether the gas phase equivalent concentrations were from an entirely different distribution from that of the gas samples. This was found not to be so. Although O_2 in water was generally lower, concentrations equivalent to 0.20ml ml^{-1} were recorded, and water samples did not account for all low O_2 concentrations recorded as can be seen in Figs. 3.13 and 3.14 on pp 121-122

The modal CO_2 concentrations (Fig. 3.7) for slurried and control plots (0.0100 - 0.0125ml ml^{-1}) were greater than for the fertilised plots (0.0050 - 0.0075ml ml^{-1}), and the median CO_2 concentrations (0.0124, 0.0126, 0.0091ml ml^{-1} for the control, slurried and fertilised plots respectively) were lower than in the previous experiment.

The CO_2 data were obviously not normal, but were satisfactorily transformed (Figs. 3.8, 3.9) by equation 2.2 which had been applied to data from the preliminary field experiment.

Transformed data were used to calculate means for each sampling occasion at each depth within each treatment, but for ease of interpretation the reverse transforms of the means are plotted in Figs. 3.10 - 3.12 and are given in Appendix 8.

In general, CO_2 peaks corresponded to O_2 troughs, but CO_2 concentrations were less than O_2 deficits and varied less from week to week (as discussed in Section 2.5). The major influences on O_2 and CO_2 concentrations (O_2 demand and moisture content) are reflected in the data. In general, O_2 concentrations varied with time in the same way at all depths but mean concentrations decreased with depth.

During the summer and autumn O_2 remained high with little variation between replicates and treatments. The soil at this time

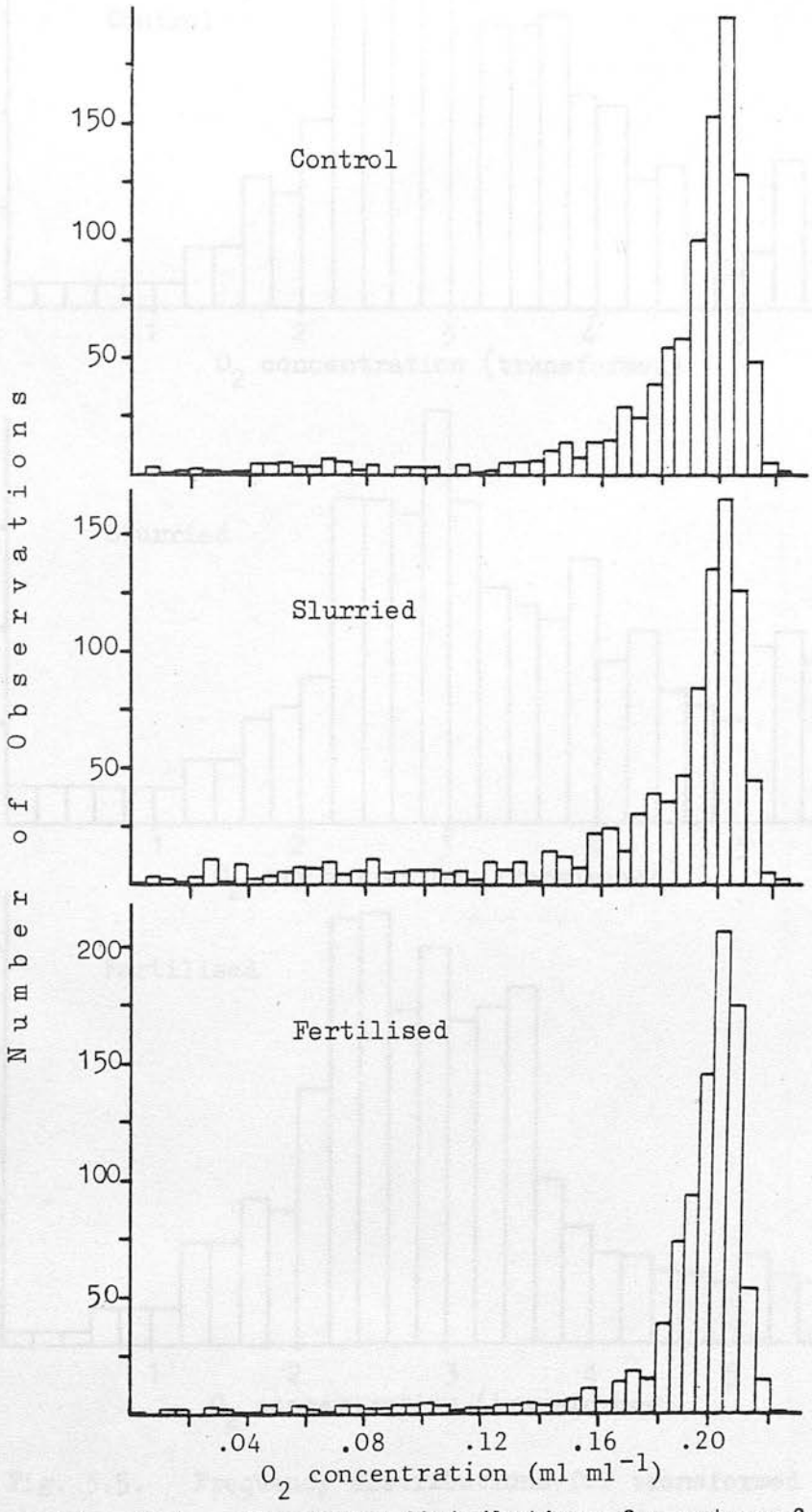


Fig. 3.4. Frequency distributions for untransformed O_2 data

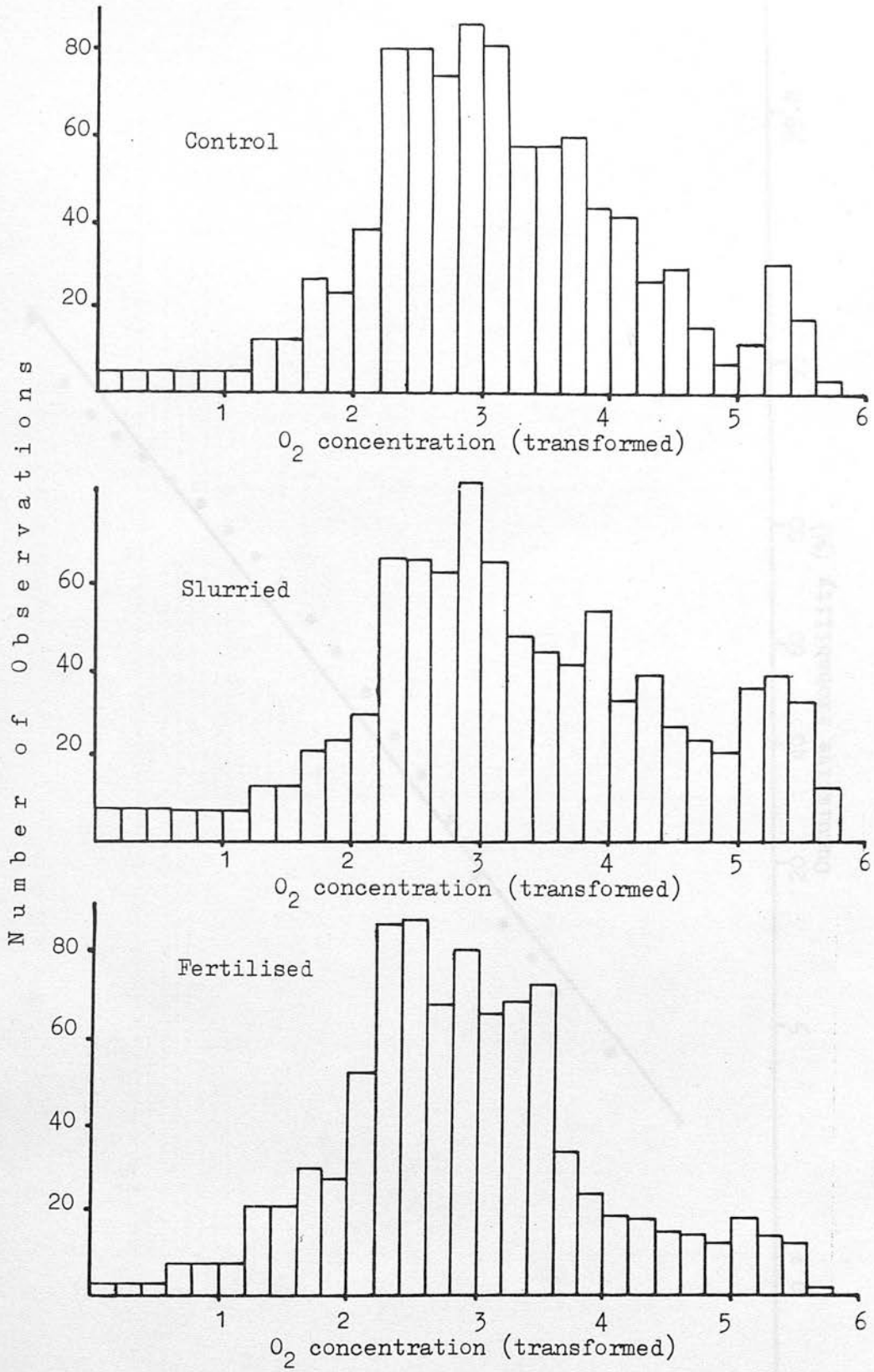


Fig. 3.5. Frequency distributions for transformed O_2 data

Note: Since concentrations in the ranges 20.75-20.85, 20.85-20.95 and 20.95-21.05 $mlml^{-1}$ (transformed values 0-1.6) could not be differentiated, the number of observations for these 3 intervals were allocated equally in the ranges 0-0.6, 0.6-1.2 and 1.2-1.4 respectively for their transformed values.

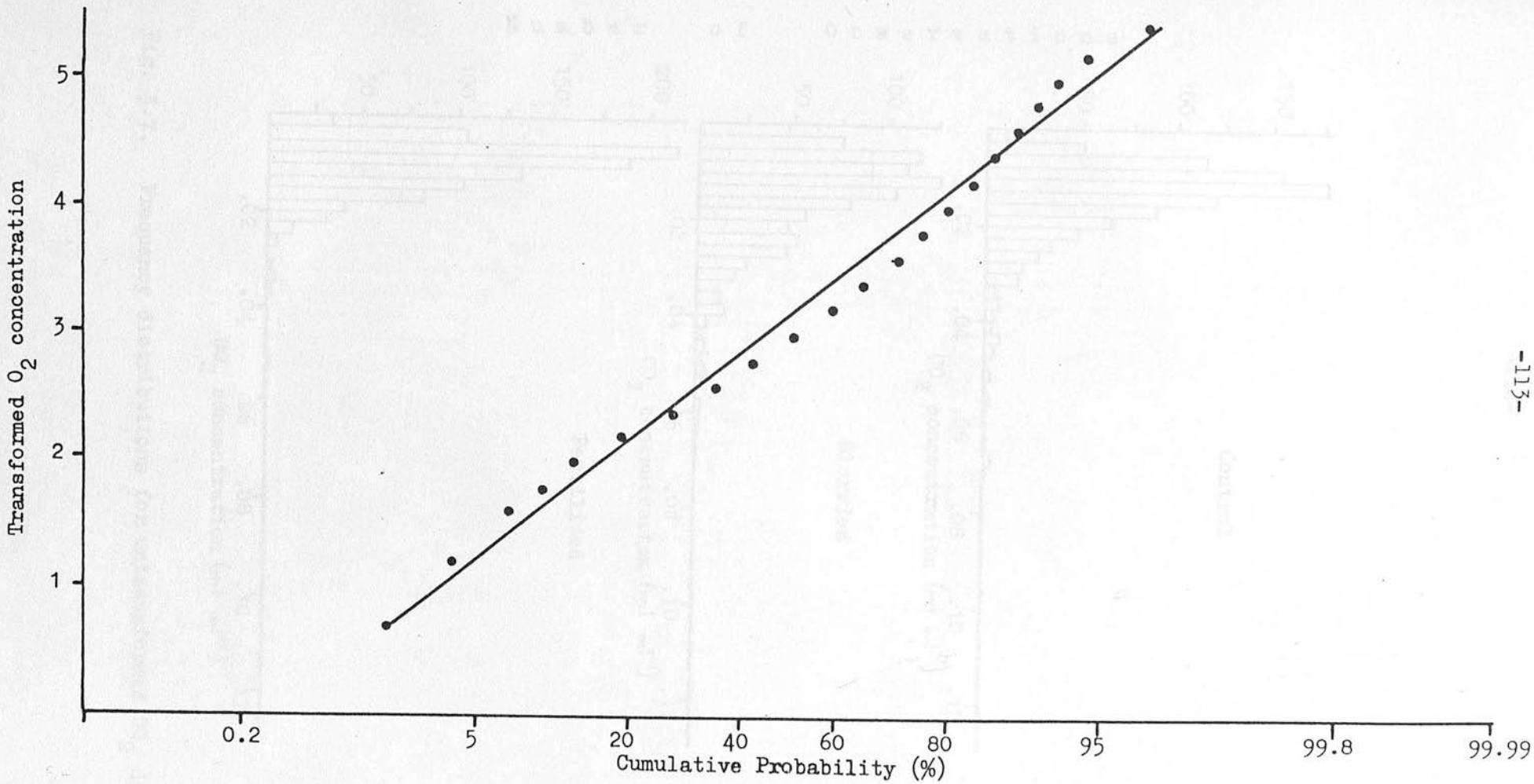


Fig. 3.6. Probability plot for combined O_2 data (transformed)

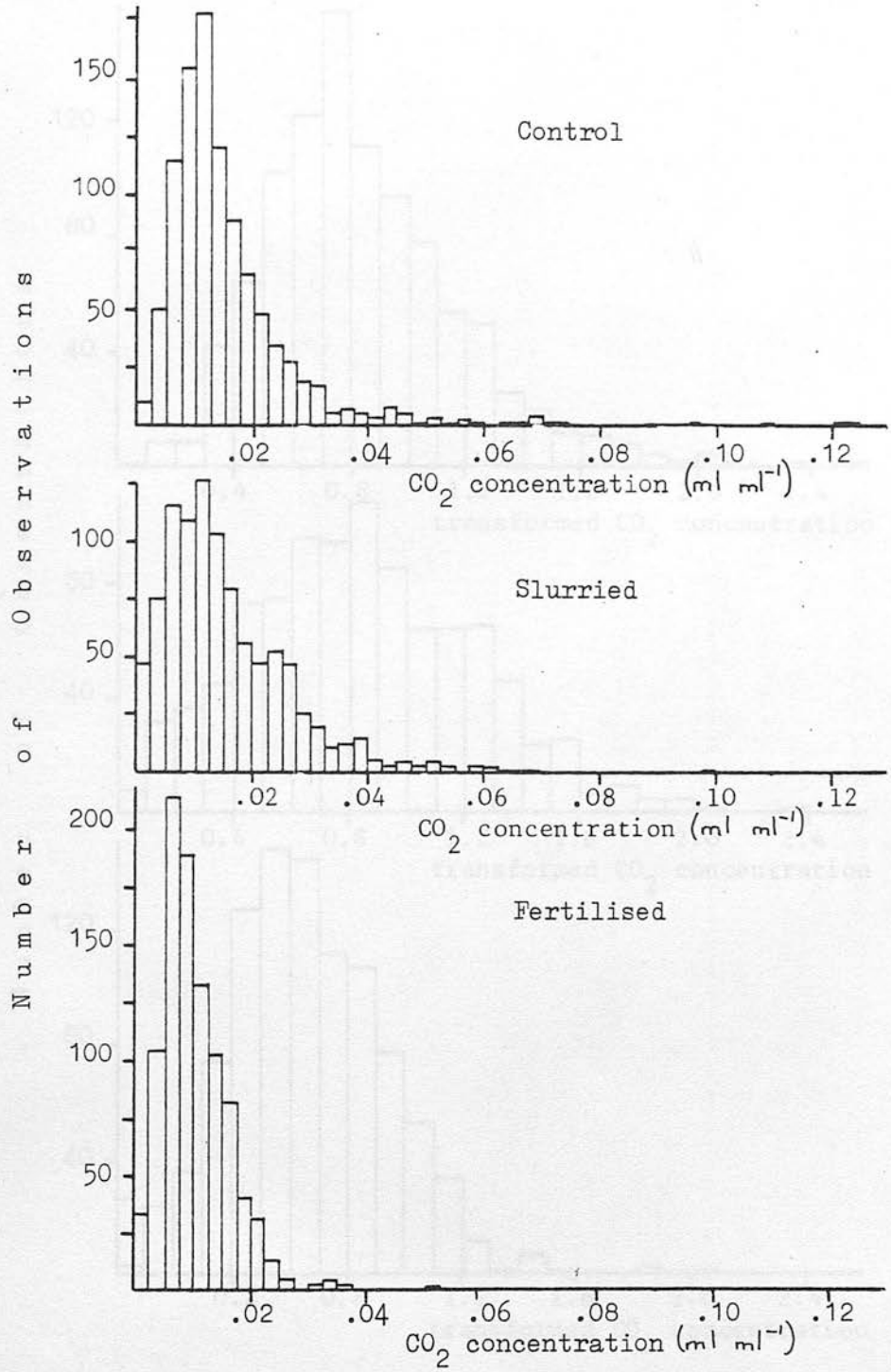


Fig. 3.7. Frequency distributions for untransformed CO_2 data

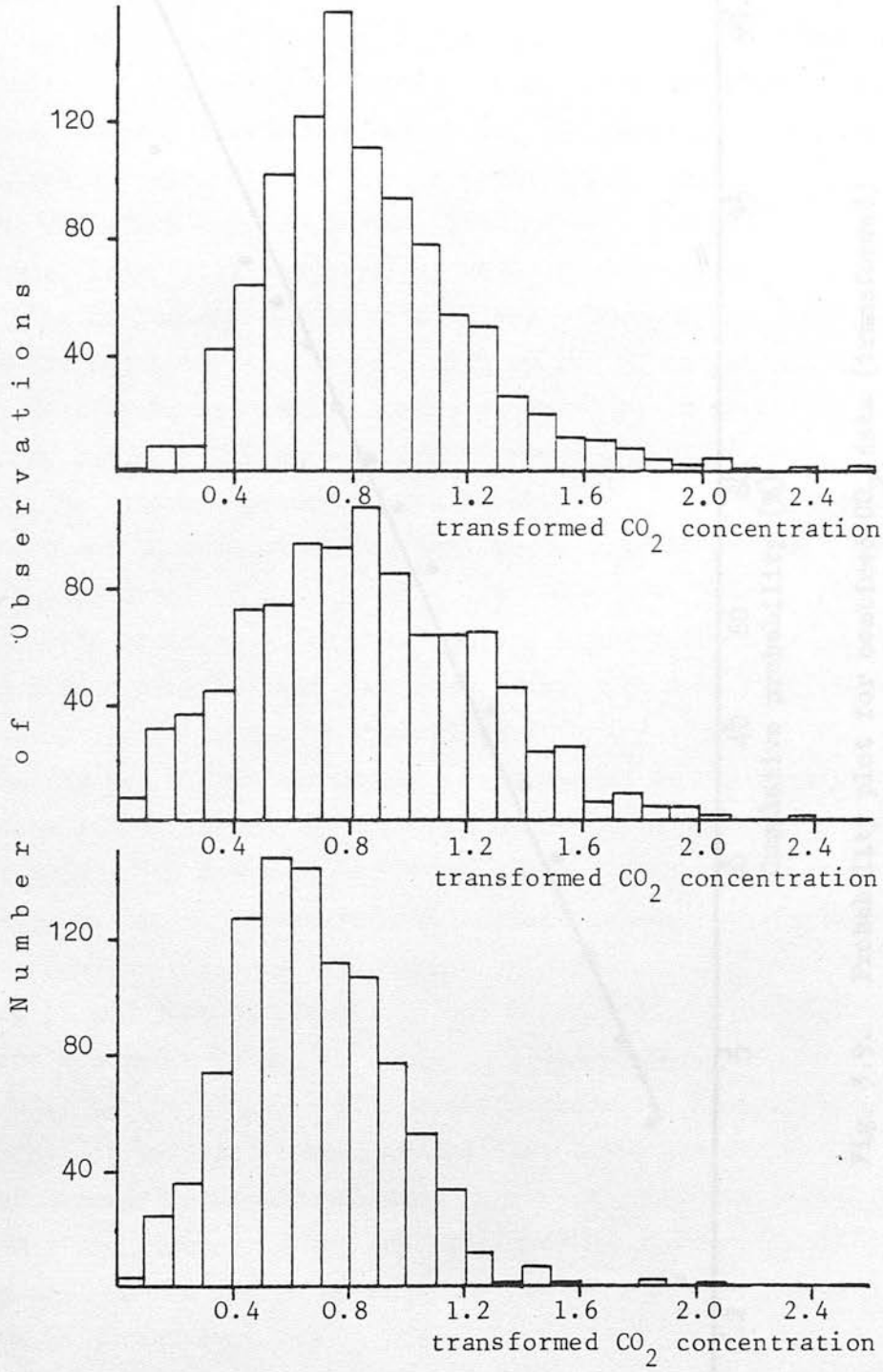


Fig. 3.8. Frequency distributions for transformed CO_2 data

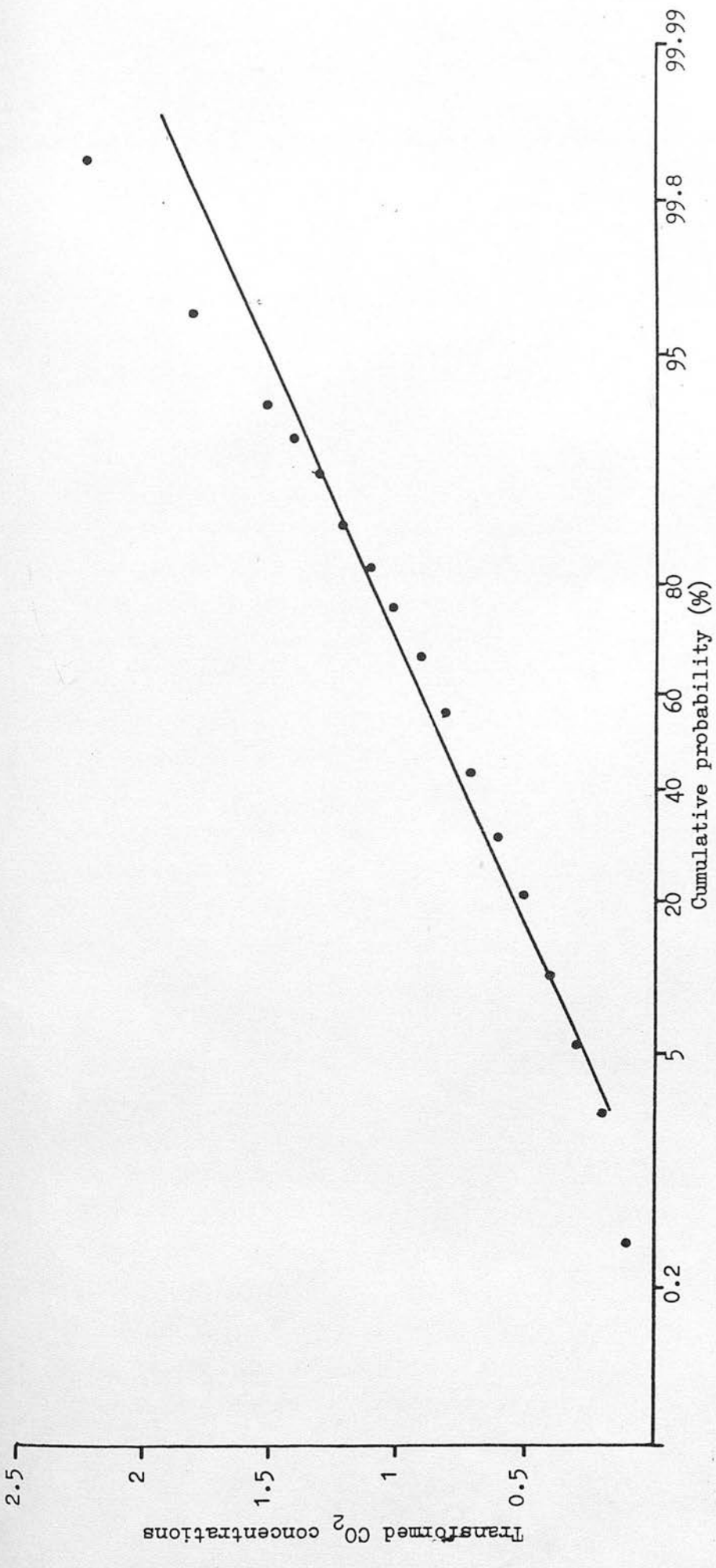


Fig. 3.9. Probability plot for combined CO_2 data (transformed)

was very dry and O_2 therefore remained high in spite of a high O_2 demand.

Following the dry summer, O_2 concentrations did not begin to fall until the beginning of October, when there was a period of heavy rainfall, whereas in the previous year, following a wet summer, O_2 concentrations were already low in early September. After early October variation between depths, treatments and replicates increased (Figs. 3.13 and 3.14) since O_2 concentrations depend largely on diffusion coefficients in wet soil which can vary greatly. Probes did not have consistently high or low O_2 concentrations during the whole winter, especially in the slurried plots where the movement of slurry may have caused variable O_2 demand.

In the winter, replicates from Blocks 1 and 2 usually had higher CO_2 and lower O_2 concentrations than those from Block 3 and more water samples were obtained from Block 1 (56) than from Block 2 (46) or Block 3 (26). In contrast to the preliminary experiment water samples were obtained from 15cm (5) and 30cm (29) as well as from 45cm (94); all those from 15 and 30cm were from Block 1.

During the winter, concentrations remained approximately constant until the end of January at 45cm and about the beginning of April at 15 and 30cm. In February and March, at a time when temperatures and therefore O_2 demand increased, O_2 concentrations fell further at 45cm even though the soil was beginning to dry out.

At 15 and 30cm O_2 concentrations increased from mid-April but at 45cm did not reach 0.19ml ml^{-1} until the end of May.

Water in the slurry applications represented no more than a single heavy fall of rain and caused only slight temporary decreases in O_2 concentration. From mid-February to May at 30cm, and during April at 45cm, slurry increased CO_2 and decreased O_2 concentrations. The spring application had no effect on O_2 at 15cm but decreased O_2 further at 30 and 45cm.

For much of the time O_2 concentrations were higher and CO_2 lower in the fertilised plots, especially at 15cm, and at 15cm control plots had lower O_2 and higher CO_2 concentrations over the entire period than other plots.

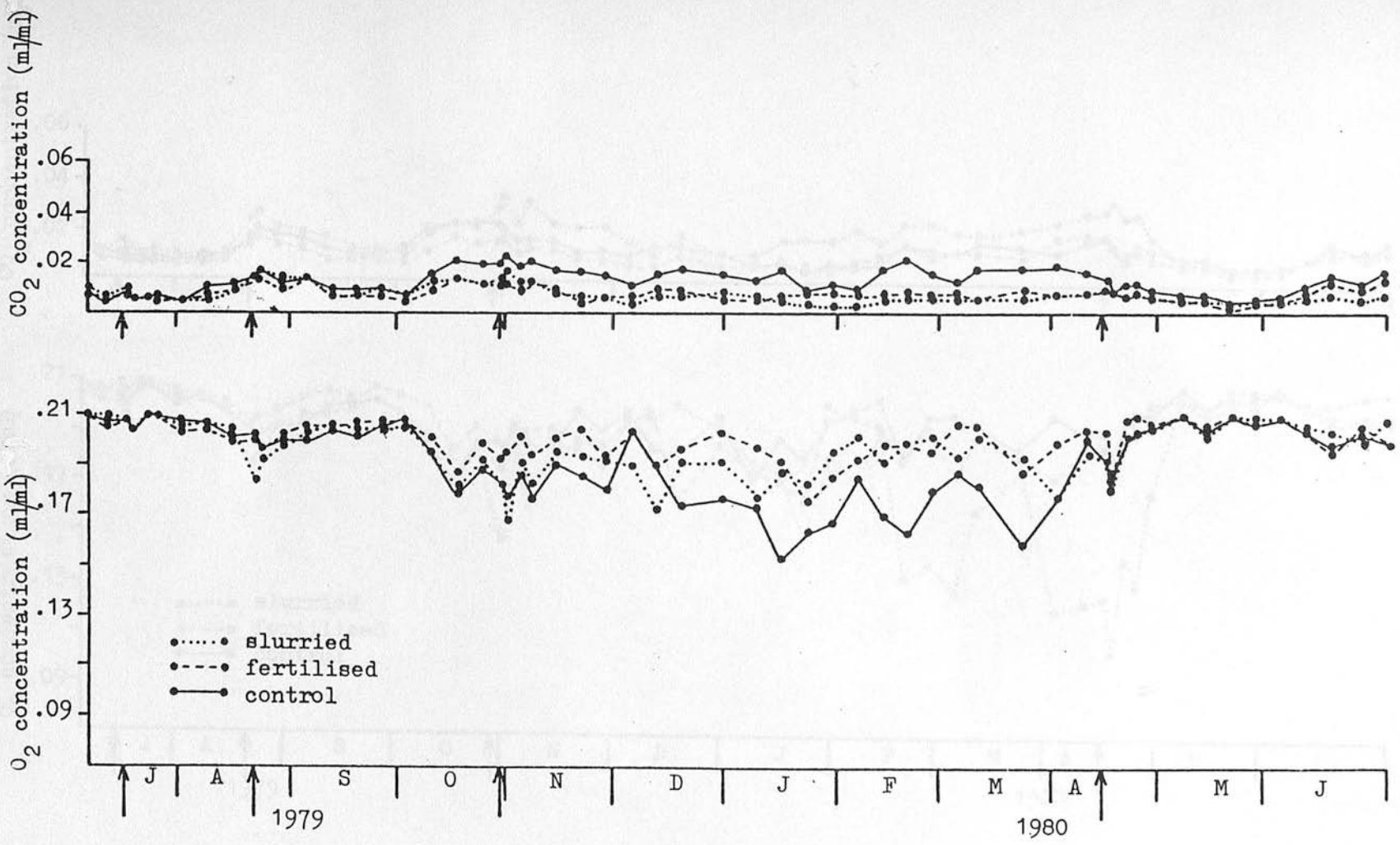


Fig. 3.10. Mean concentrations of O_2 and CO_2 at the 15cm depth (arrows indicate fertiliser and slurry applications)

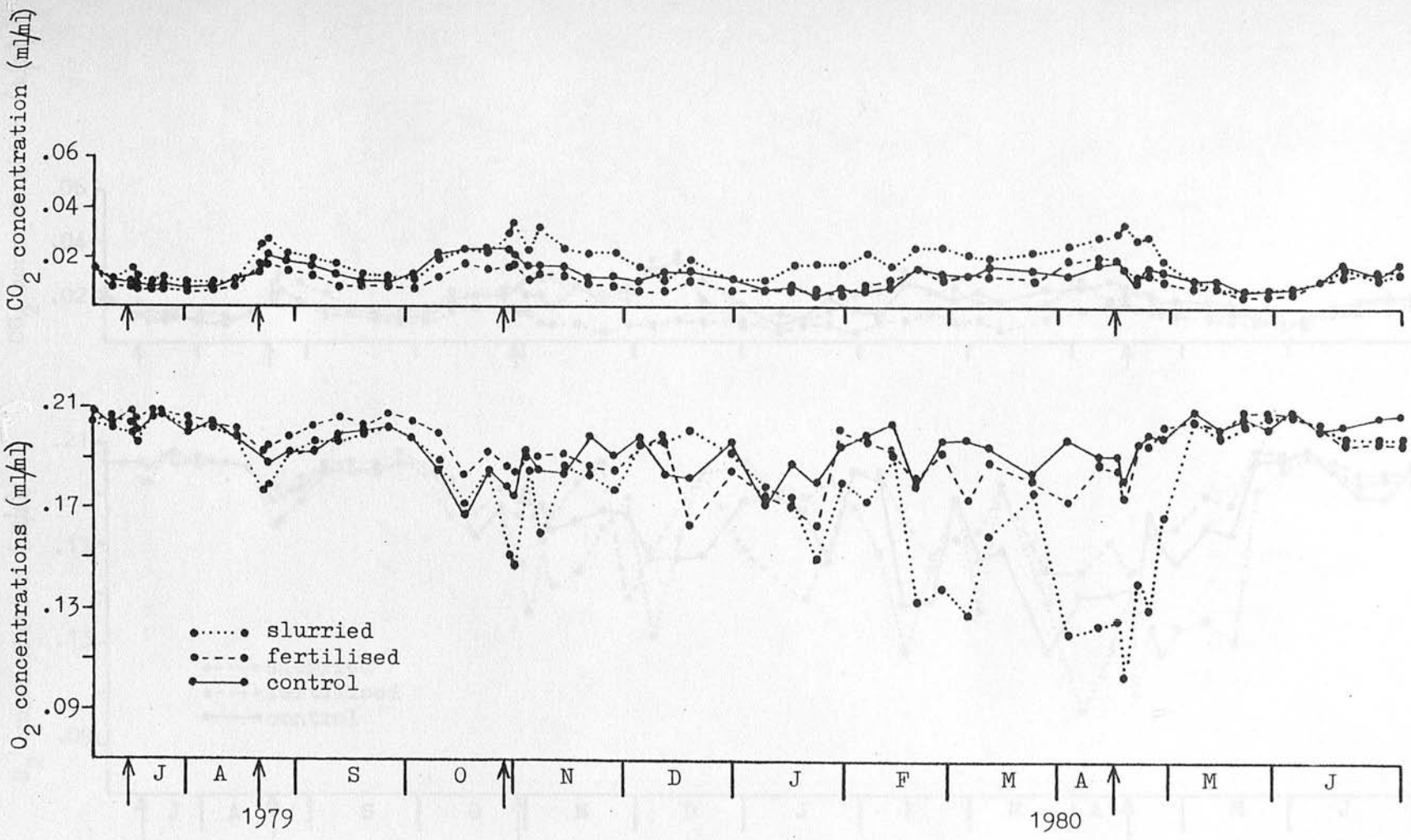


Fig. 3.11. Mean concentrations of CO_2 and O_2 at the 30cm depth (arrows indicate applications of slurry and fertiliser)

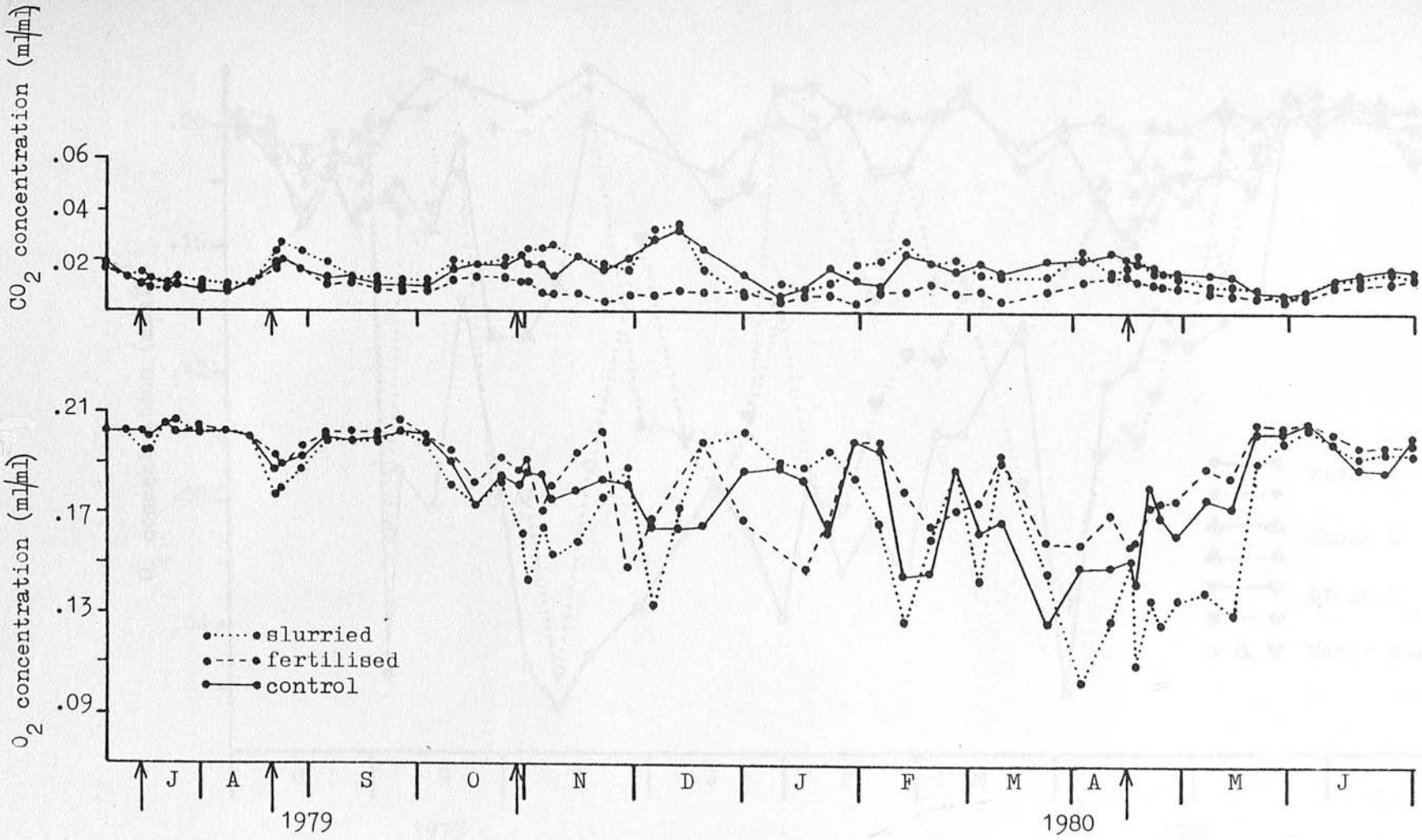


Fig. 3.12. Mean concentrations of CO_2 and O_2 at the 45cm depth (arrows indicate applications of slurry and fertiliser)

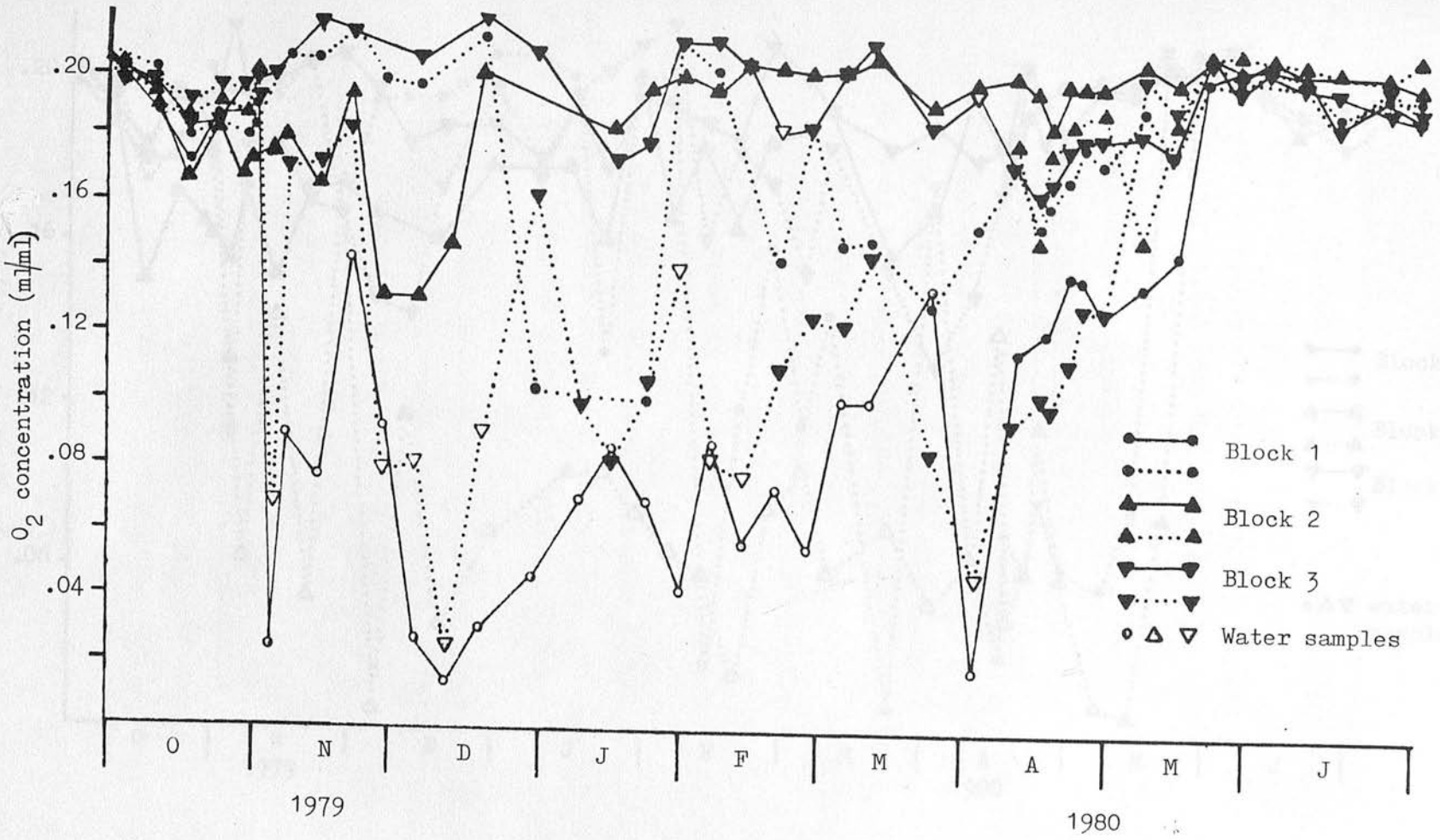


Fig. 3.13. O_2 concentrations from replicate probes (fertilised plot at 45cm)

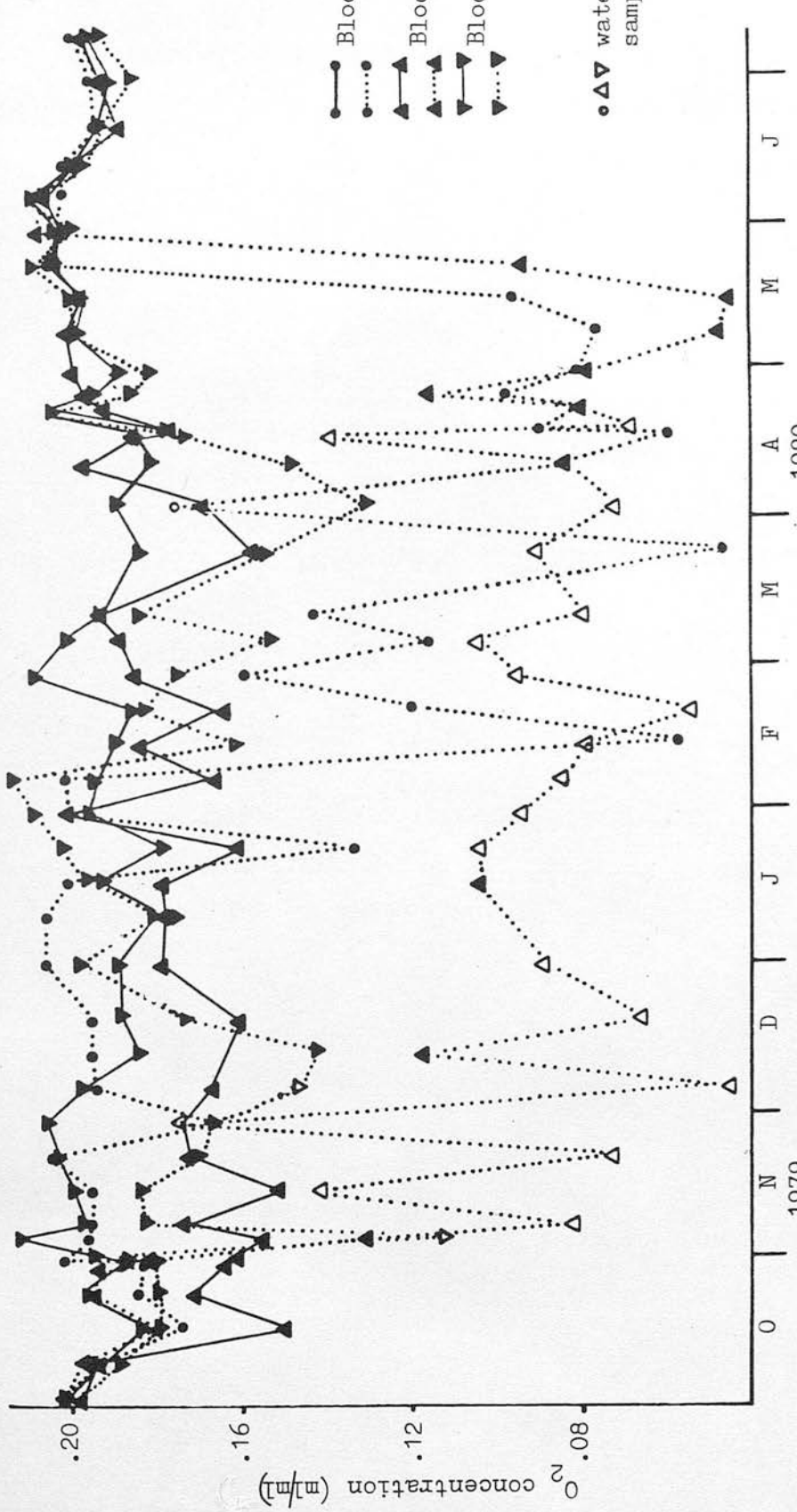


Fig. 3.14. O_2 concentrations from replicate probes (control plot at 45cm)

In comparison with the preliminary experiment, O_2 concentrations were higher and CO_2 lower in spite of similar overall rainfall and higher temperatures during the winter, probably because the rainfall in the autumn and spring was lower during the second experiment and there was no long period when the surface soil was frozen.

3.7. Nitrous Oxide Concentrations

3.7.1. Frequency Distribution of N_2O Concentrations

Most observed N_2O concentrations were below 2×10^{-6} ml ml^{-1} (Fig. 3.15), the median values (0.8×10^{-6}, 1.1×10^{-6} and 0.9×10^{-6} mlml^{-1} for the control, slurried and fertilised plots respectively) being much lower than those for the preliminary experiment. A few observations were below 0.3×10^{-6} ml ml^{-1}, but this may be accounted for by experimental error since the ECD detector had to be set to measure a wide range of N_2O concentrations (i.e. using the pulse spacing of 150μs), reducing the accuracy of measurements at the lowest concentrations.

The range of values show clear treatment differences: the highest values recorded for the three treatments being 33.5, 150 and 696×10^{-6} ml ml^{-1} for the control, slurried and fertilised plots respectively.

The transform used previously (Equation 2.3) did not give a normal distribution, but if values below 0.3×10^{-6} ml ml^{-1} were omitted an approximately normal distribution (Figs. 3.16 and 3.17) was obtained from the transform:

$$Z' = 5 + \ln (Z - 0.29) \qquad 3.2$$

where Z = measured N_2O concentration (ml ml^{-1} $\times 10^6$)
Z' = transformed N_2O concentration

The constant, 5, was added to avoid negative values. The constant, 0.29, is in effect a correction term for the "background" N_2O.

3.7.2. Regression of N_2O on O_2

Scatter diagrams of N_2O against O_2 showed a clear relationship only when transformed values were used for both N_2O and O_2 (an example is given in Fig. 3.18). At no depth and in no treatment was there

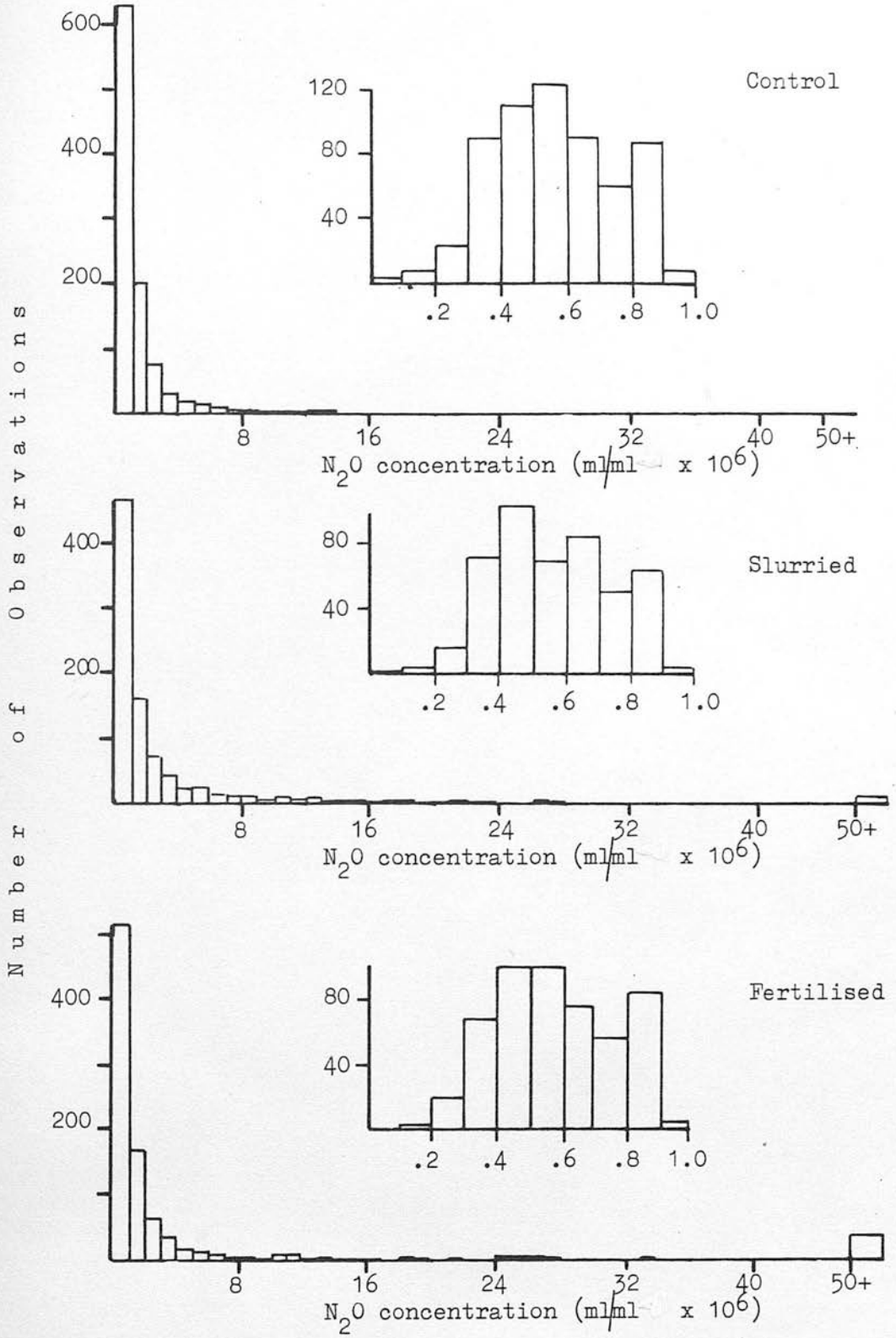


Fig. 3.15. Frequency distributions for untransformed N_2O data

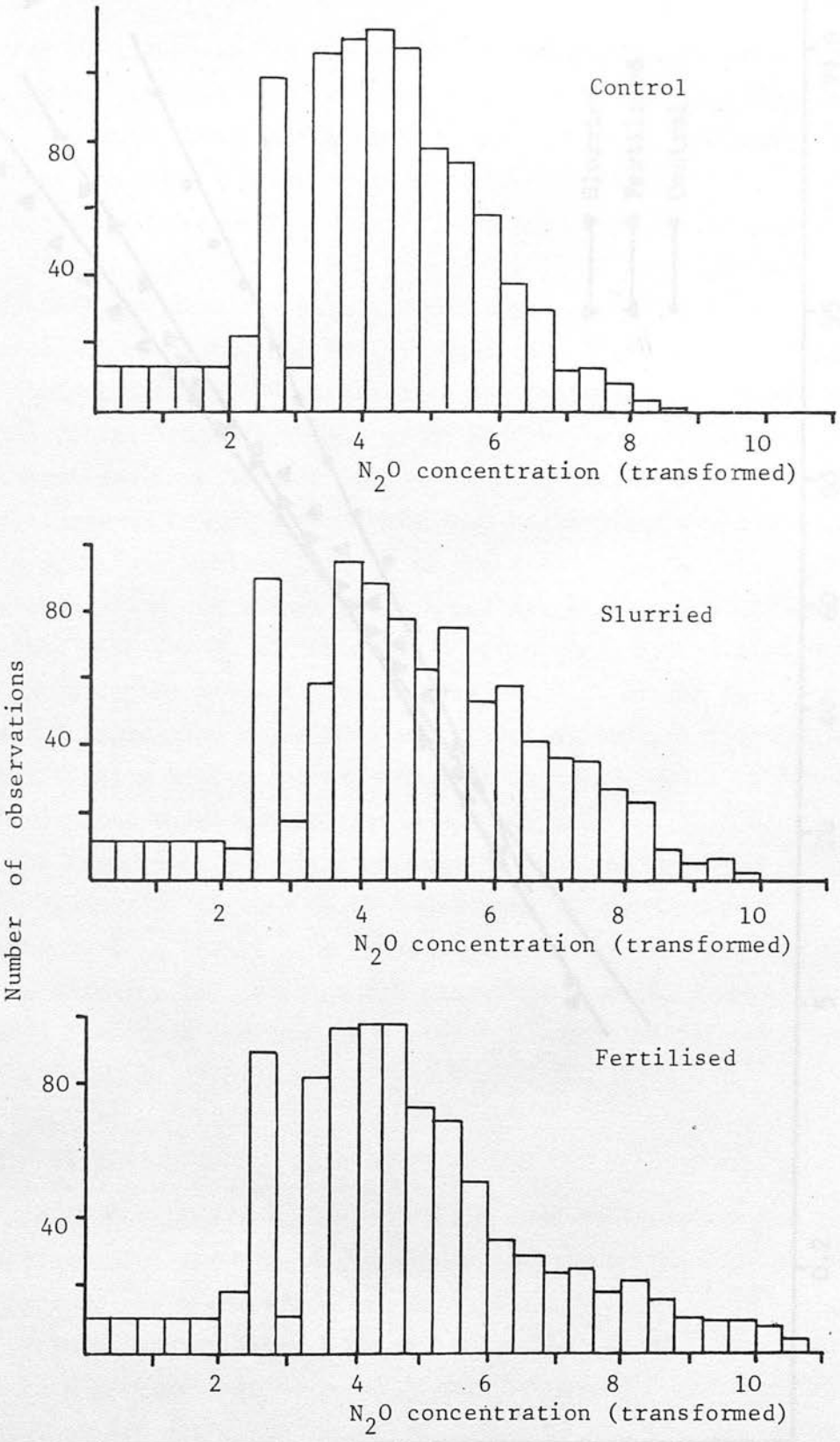


Fig. 3.16. Frequency distributions for transformed N_2O data

Note: Since concentrations in the range of 0.3 to 0.35 x 10^{-6} ml ml^{-1} (transformed values 0-2) could not be differentiated, such concentrations have been allocated equally in the range 0-2.

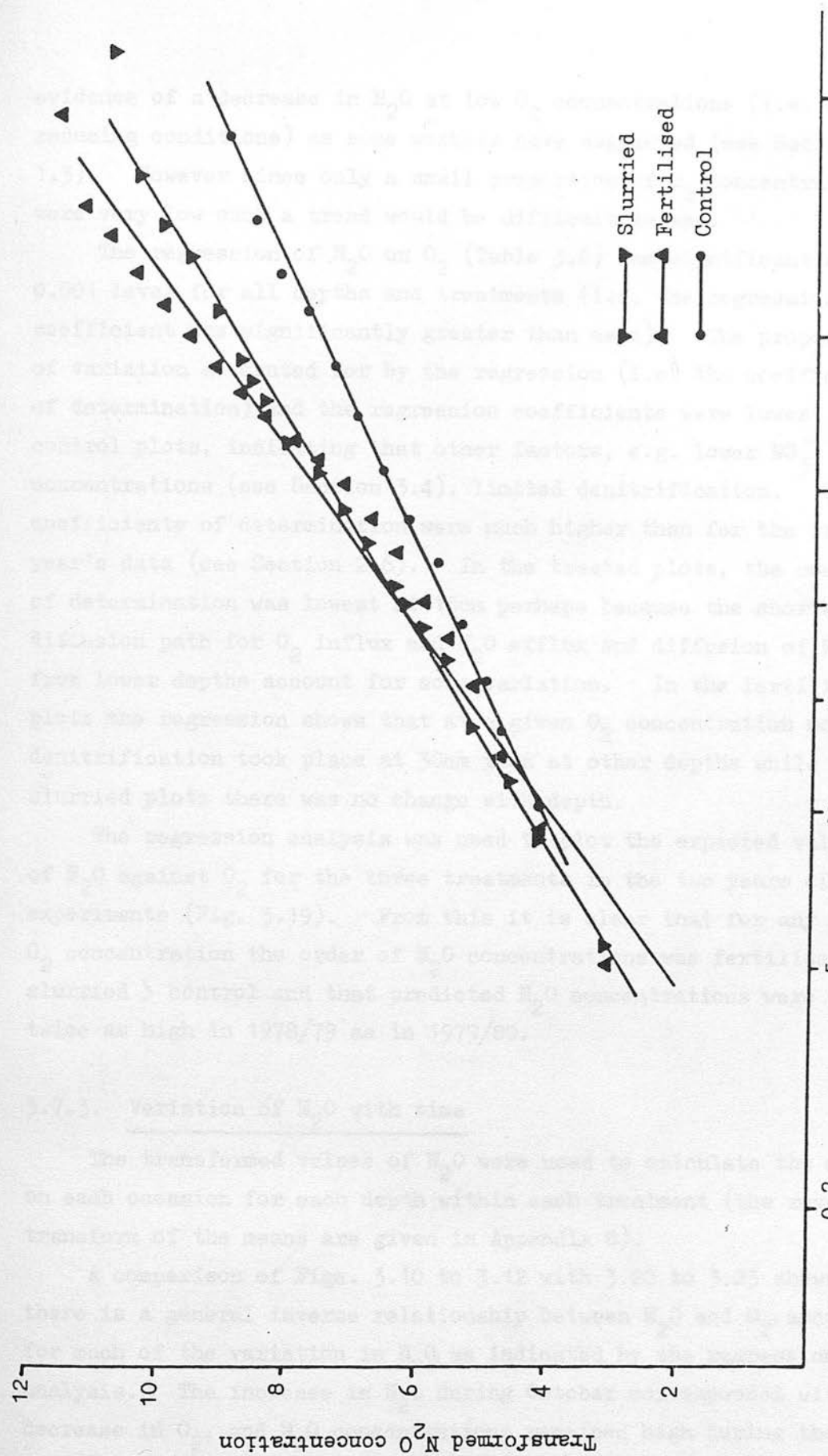


Fig. 3.17. Probability plots for transformed N_2O data

evidence of a decrease in N_2O at low O_2 concentrations (i.e. severely reducing conditions) as some workers have suggested (see Section 1.5. 1.3). However since only a small proportion of O_2 concentrations were very low such a trend would be difficult to see.

The regression of N_2O on O_2 (Table 3.6) was significant at the 0.001 level for all depths and treatments (i.e. the regression coefficient was significantly greater than zero). The proportion of variation accounted for by the regression (i.e. the coefficient of determination) and the regression coefficients were lowest in the control plots, indicating that other factors, e.g. lower NO_3^- concentrations (see Section 3.4), limited denitrification. The coefficients of determination were much higher than for the previous year's data (see Section 2.6). In the treated plots, the coefficient of determination was lowest at 15cm perhaps because the shorter diffusion path for O_2 influx and N_2O efflux and diffusion of N_2O from lower depths account for some variation. In the fertilised plots the regression shows that at a given O_2 concentration more denitrification took place at 30cm than at other depths while in slurried plots there was no change with depth.

The regression analysis was used to plot the expected values of N_2O against O_2 for the three treatments in the two years of experiments (Fig. 3.19). From this it is clear that for any given O_2 concentration the order of N_2O concentrations was fertilised > slurried > control and that predicted N_2O concentrations were over twice as high in 1978/79 as in 1979/80.

3.7.3. Variation of N_2O with time

The transformed values of N_2O were used to calculate the means on each occasion for each depth within each treatment (the reverse transform of the means are given in Appendix 8).

A comparison of Figs. 3.10 to 3.12 with 3.20 to 3.23 shows that there is a general inverse relationship between N_2O and O_2, accounting for much of the variation in N_2O, as indicated by the regression analysis. The increase in N_2O during October corresponded with the decrease in O_2, and N_2O concentrations remained high during the winter, decreasing when O_2 returned to ambient concentrations. However,

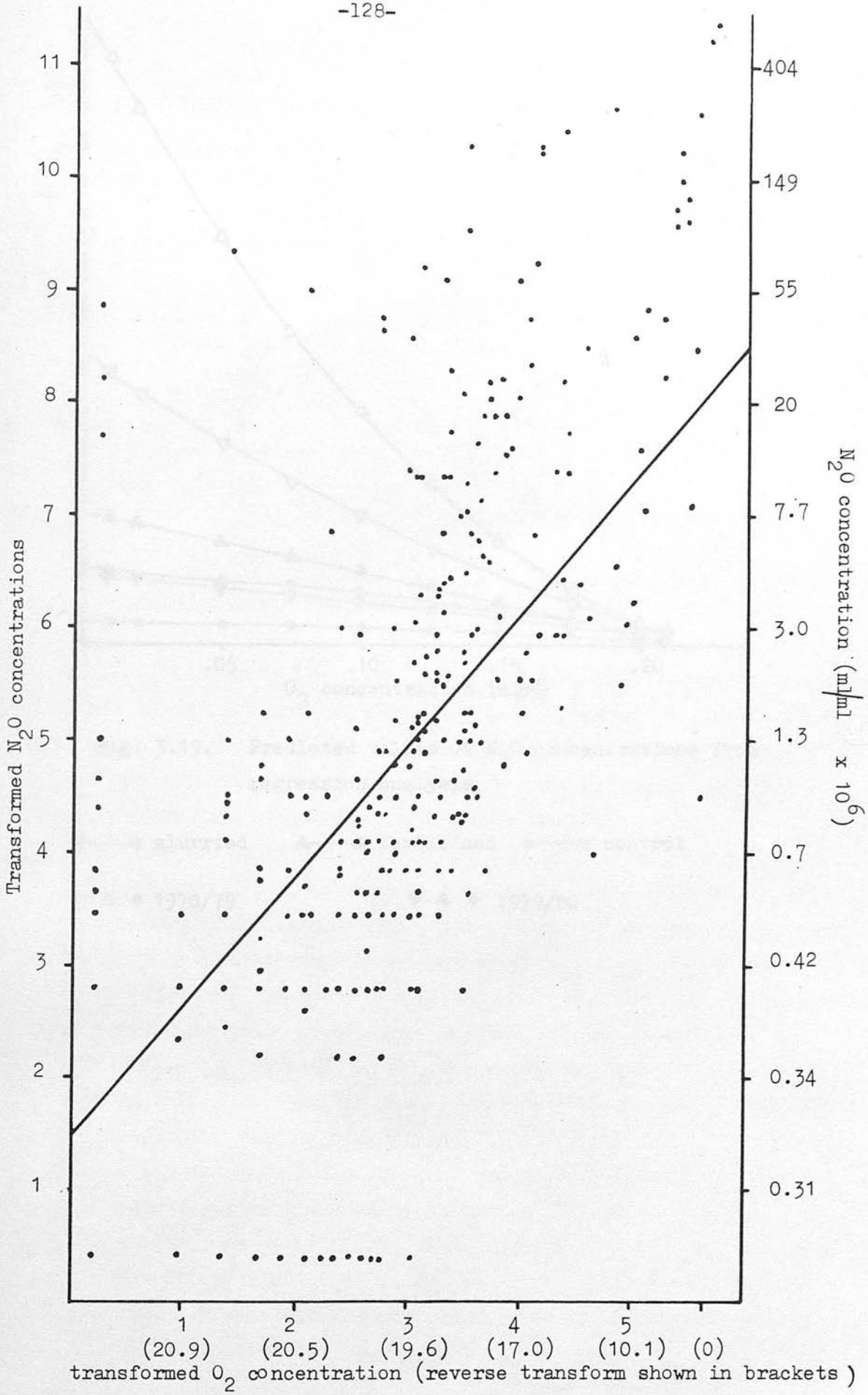


Fig. 3.18. Scatter diagram of N_2O against O_2 concentrations (fertilised plot at 30cm) showing the regression line

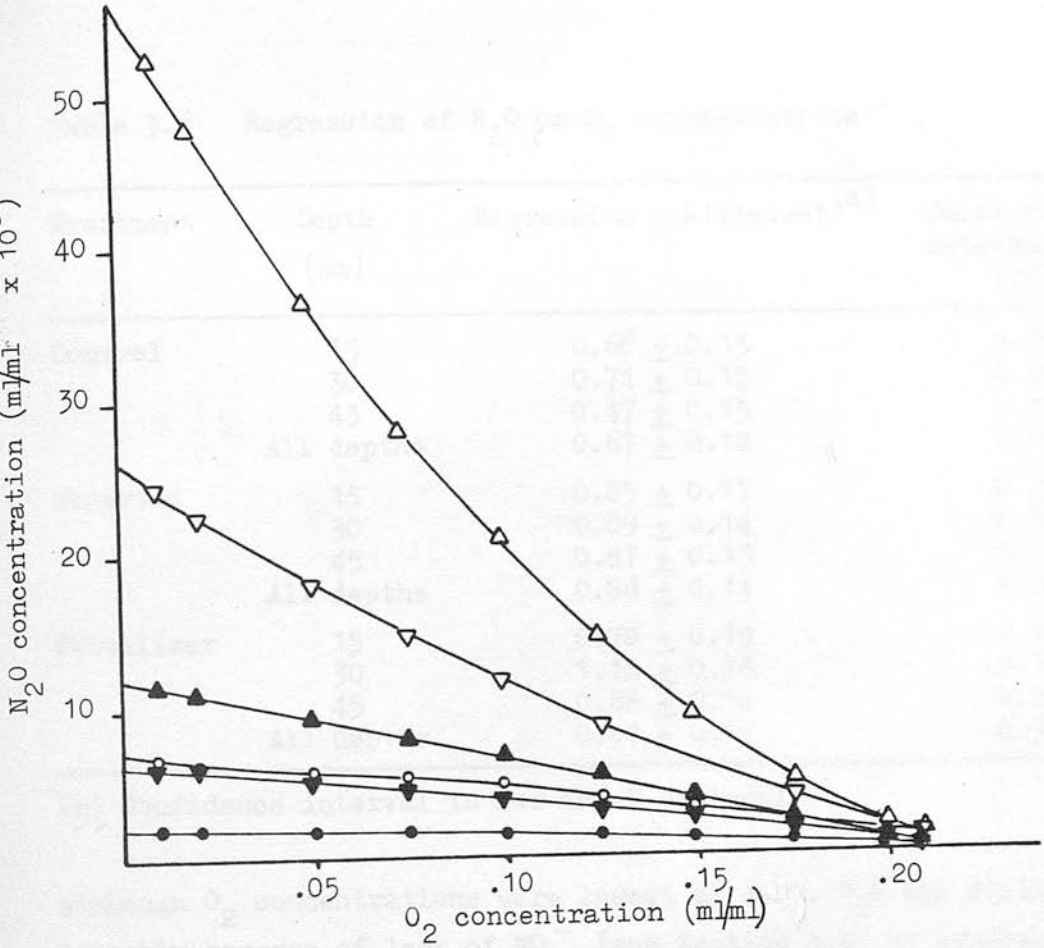


Fig. 3.19. Predicted values of N_2O concentrations from regression analysis

▼—▼ slurried ▲—▲ fertilised ●—● control

▽ △ ○ 1978/79 ▼ ▲ ● 1979/80

Table 3.6 Regression of N_2O on O_2 concentrations

Treatment	Depth (cm)	Regression coefficient[a]	Coefficient of determination (r^2)
Control	15	0.66 ± 0.13	0.23
	30	0.71 ± 0.15	0.21
	43	0.47 ± 0.15	0.11
	All depths	0.67 ± 0.12	0.19
Slurried	15	0.85 ± 0.17	0.24
	30	0.89 ± 0.14	0.33
	45	0.87 ± 0.13	0.34
	All depths	0.88 ± 0.14	0.32
Fertiliser	15	0.78 ± 0.19	0.18
	30	1.18 ± 0.18	0.34
	45	0.88 ± 0.14	0.33
	All depths	0.97 ± 0.17	0.30

(a) Confidence interval is for the 0.05 level

although O_2 concentrations were lowest at 45cm, N_2O was similar at 45cm and 30c probably because of lack of NO_3^- (see Section 3.4) or available carbon substrates at 45cm. As with O_2 and CO_2 (see Section 3.6) variation in N_2O concentrations between replicates and treatments increased after October (cf. Figs. 3.13 and 3.14 with 3.23).

Neither the application of slurry nor fertiliser affected N_2O concentrations in July, presumably because the soil at this time was fully aerobic. The August application of slurry did cause a slight increase in N_2O, reflecting the slight decrease in O_2.

The effect of the October applications of slurry and fertiliser, when O_2 concentrations were lower, was greater and lasted over the entire winter, as indicated by NO_3^- concentrations (see Section 3.4) as well as N_2O. At 15cm N_2O was increased by slurry and fertiliser until mid-January, and then decreased in all plots. At 30cm slurry increased N_2O only until the end of November, even though O_2 remained low, whereas fertiliser increased N_2O right through the winter. At 45cm both slurry and fertiliser, but especially fertiliser, increased N_2O concentrations right through the winter. After April, both at 30 and 45cm, N_2O concentrations decreased in fertilised and control plots as O_2 increased, but increased again in the slurried plots since

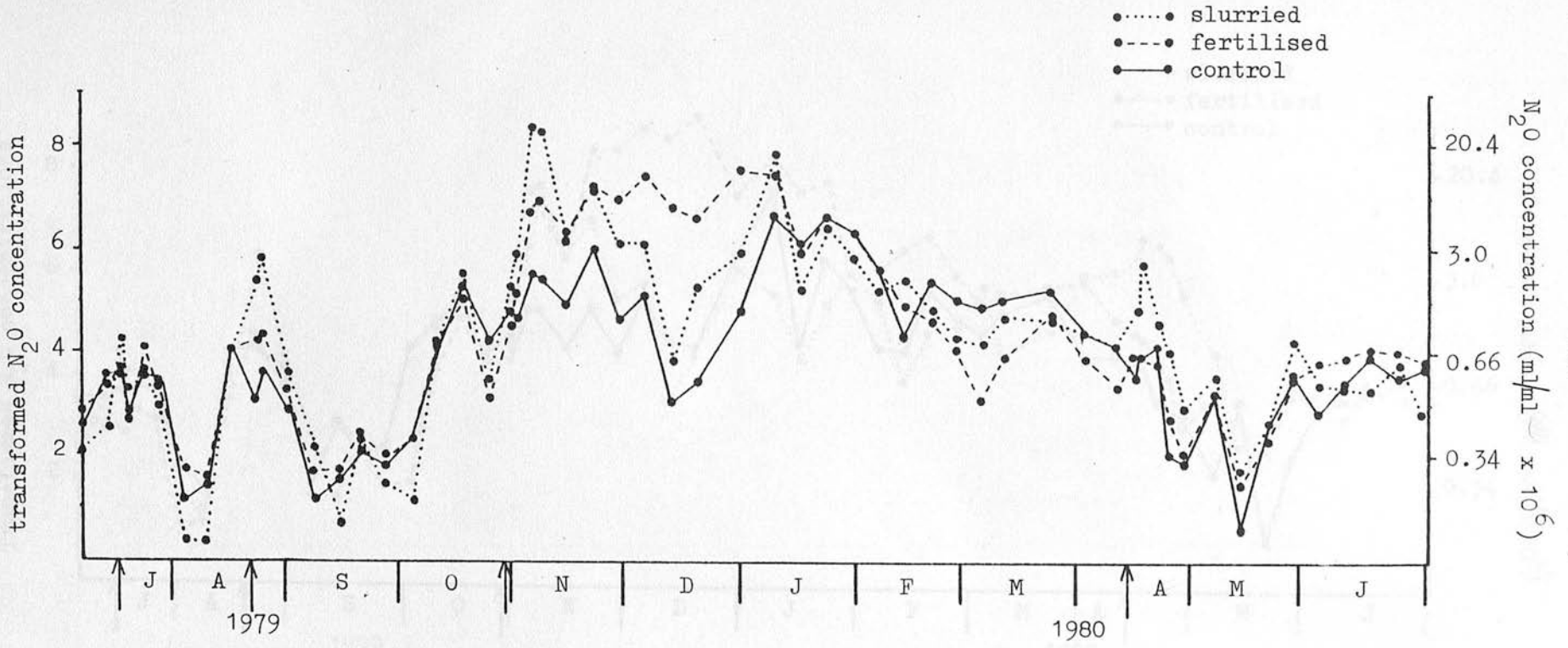


Fig. 3.20. Mean concentrations of N_2O at the 15cm depth
(arrows indicate applications of slurry and fertiliser)

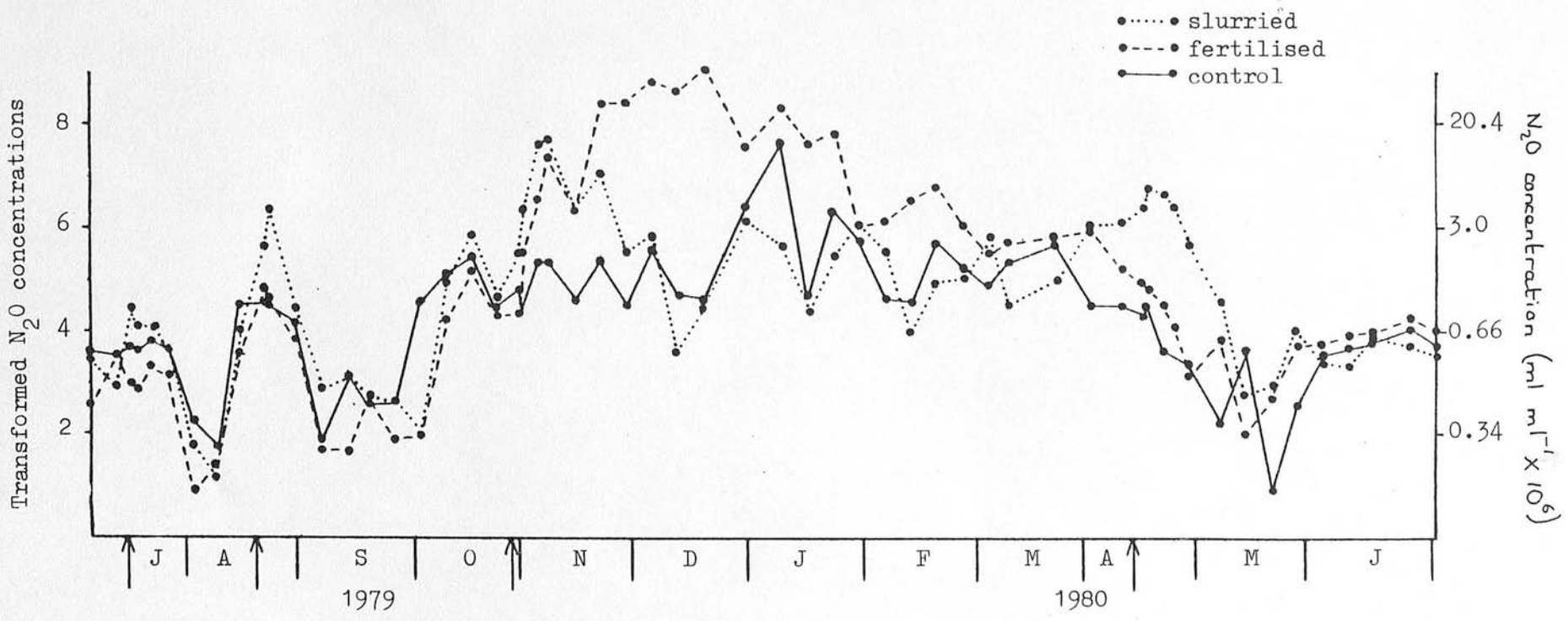


Fig. 3.21. Mean N_2O concentrations at the 30cm depth
(arrows indicate slurry and fertiliser applications)

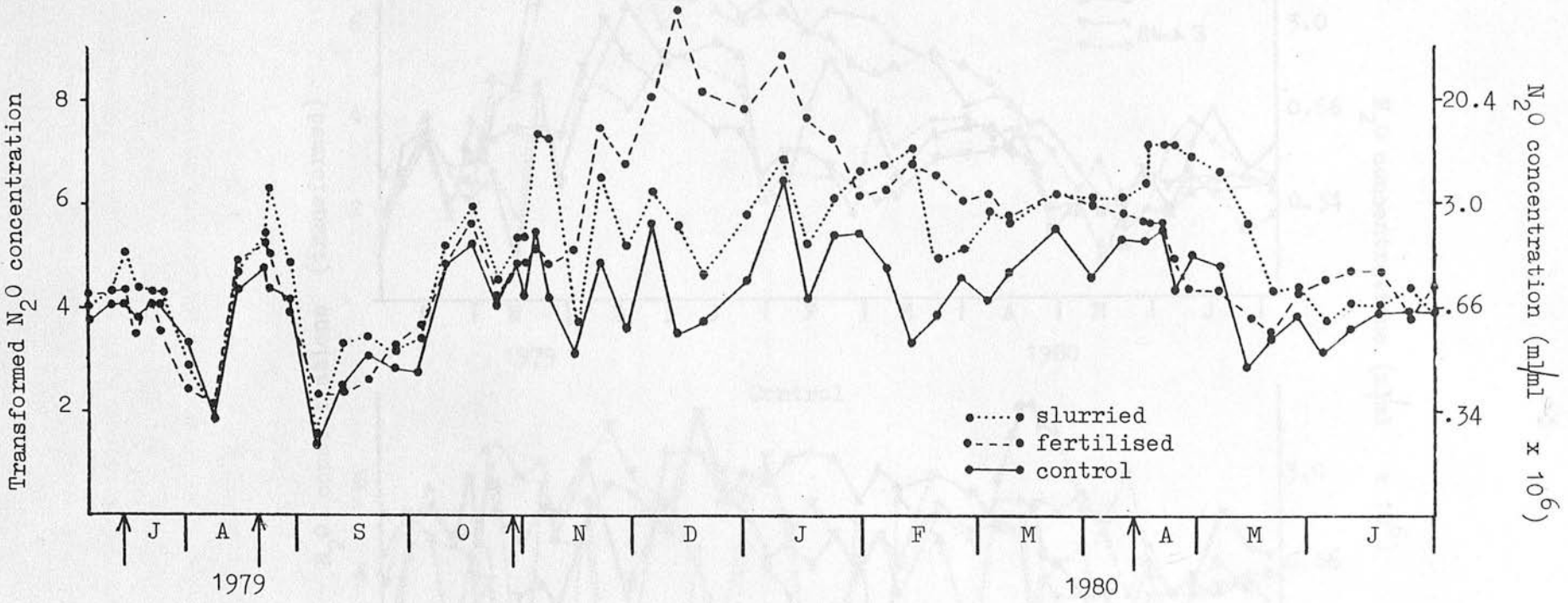


Fig. 3.22. Mean N_2O concentrations at the 45cm depth
(arrows indicate applications of slurry and fertiliser)

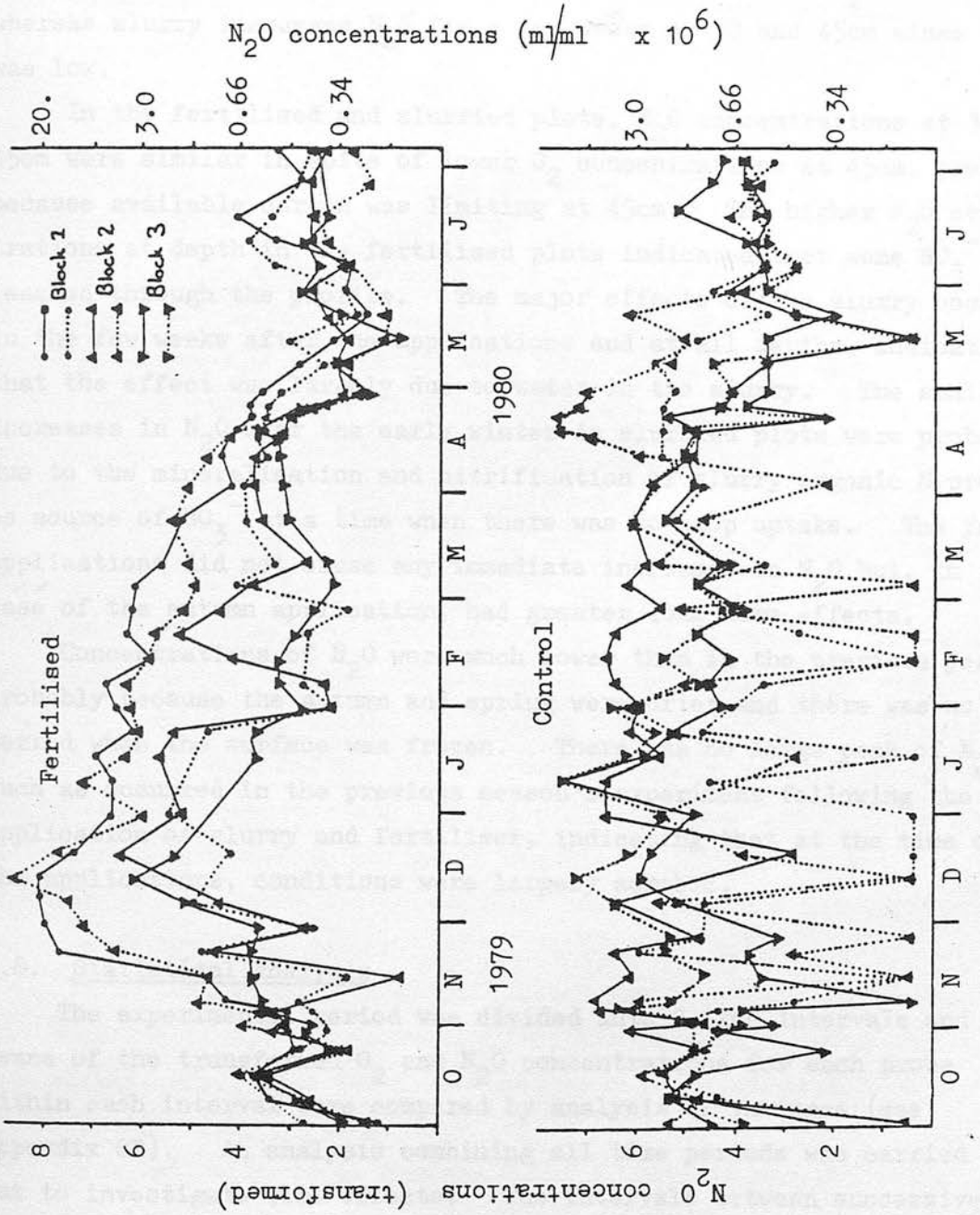


Fig. 3.23. Concentrations of N_2O from replicate probes (fertilised and control plots at the 45cm depth)

O_2 remained low.

The fertiliser application in April did not affect N_2O concentration, whereas slurry increased N_2O for a few weeks at 30 and 45cm since O_2 was low.

In the fertilised and slurried plots, N_2O concentrations at 30 and 45cm were similar in spite of lower O_2 concentrations at 45cm, probably because available carbon was limiting at 45cm. The higher N_2O concentrations at depth in the fertilised plots indicated that some NO_3^- had leached through the profile. The major effects of the slurry occurred in the few weeks after the applications and at all depths, indicating that the effect was largely due to water in the slurry. The small increases in N_2O over the early winter in slurried plots were probably due to the mineralisation and nitrification of slurry organic N providing as source of NO_3^- at a time when there was no crop uptake. The fertiliser applications did not cause any immediate increases in N_2O but, in the case of the autumn application, had greater long term effects.

Concentrations of N_2O were much lower than in the previous year, probably because the autumn and spring were drier and there was no long period when the surface was frozen. There was no large peak of N_2O, such as occurred in the previous season's experiment following the application of slurry and fertiliser, indicating that at the time of the applications, conditions were largely aerobic.

3.8. Statistical Analysis

The experimental period was divided into 8 time intervals and the means of the transformed O_2 and N_2O concentrations for each probe within each interval were compared by analysis of variance (see Appendix 8B). An analysis combining all time periods was carried out to investigate time effects. The intervals between successive periods were long enough so that the mean concentrations for each period were independent of means for other periods. Analysis of variance was also carried out on probe means for the whole period.

The data were analysed as a split plot, randomised block design, with the treatments applied to 9 main plots, and depths considered as sub-plots. In the overall analysis, time was considered as a sub-sub-plot.

The time intervals were:

(1) 17th July - 19th July - immediately following the 1st application
(2) 23rd July - 15th August - between 1st and 2nd application
(3) 22nd August - 24th August - immediately following the 2nd application

(4) 30th August - 24th October - between the 2nd and 3rd application
(5) 31st October - 1st November - immediately following the 3rd application
(6) 5th November - 11th April - between the 3rd and 4th application
(7) 16th April - 30th April - immediately following the 4th application
(8) 8th May - 2nd July - after the 4th application

The means for the 8 periods are given in Tables 3.7 and 3.8 and illustrated in Figs. 3.24 and 3.25.

Differences between blocks were significant only once for O_2, and for three periods for N_2O, when twice block 2 and only once block 3 had the lowest N_2O concentrations. The analysis of means over the whole period showed that block effects were insignificant for both N_2O and O_2. Therefore the trends noted in the previous season (Section 2.5 and 2.6) were not significant.

Apart from during period 5, O_2 concentrations always decreased with depth, the difference between 15 and 30cm being significant more frequently (5 times) than between 30 and 45cm (3 times): this was confirmed also by the overall analysis. Correspondingly, except in Periods 5 and 6 i.e. during the winter, N_2O concentrations increased significantly with depth. In the overall analysis for N_2O only the difference between 15cm and other depths was significant. The depth x treatment interaction was never significant for N_2O or O_2, indicating that there was no evidence that treatments had different effects at the three depths.

In periods 1 and 3, i.e. just after the first and second applications, the increase in N_2O and decrease in O_2 due to slurry, though small, was significant. The fertiliser had no significant effect on N_2O or O_2 during these periods.

Treatment effects in Periods 2 and 4 (between applications) were not significant.

In period 5, after the 3rd application, slurry significantly decreased O_2 and increased N_2O but the fertiliser had no effect.

During the winter (Period 6) the differences between treatments for neither O_2 nor N_2O were significant, because variation between replicates was very high.

Following the final application (Period 7) slurry decreased O_2 and increased N_2O significantly but there were no significant

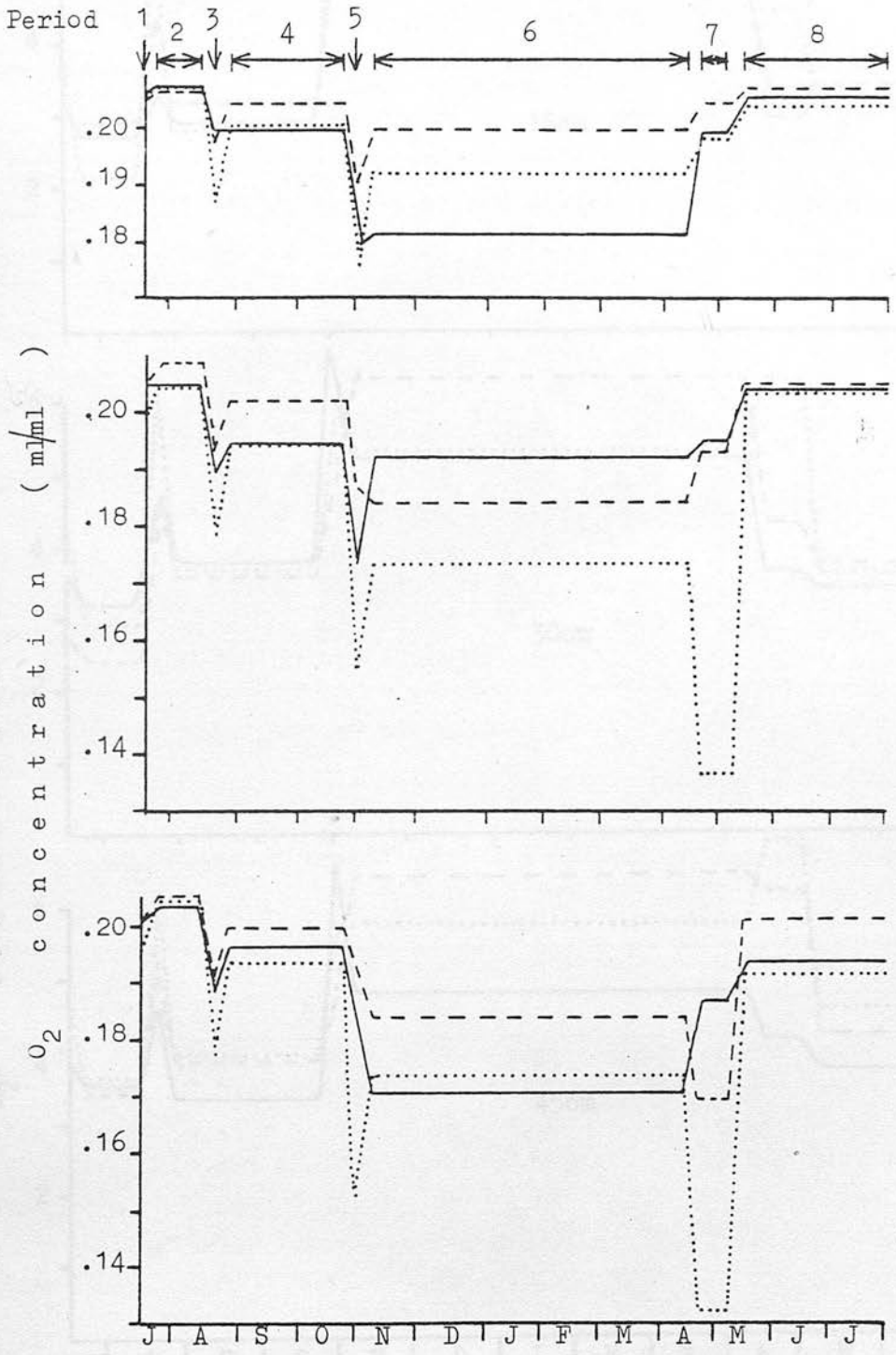


Fig. 3.24. Means of O_2 concentration for depths and treatments during 8 periods
·····slurried --- fertilised—— control

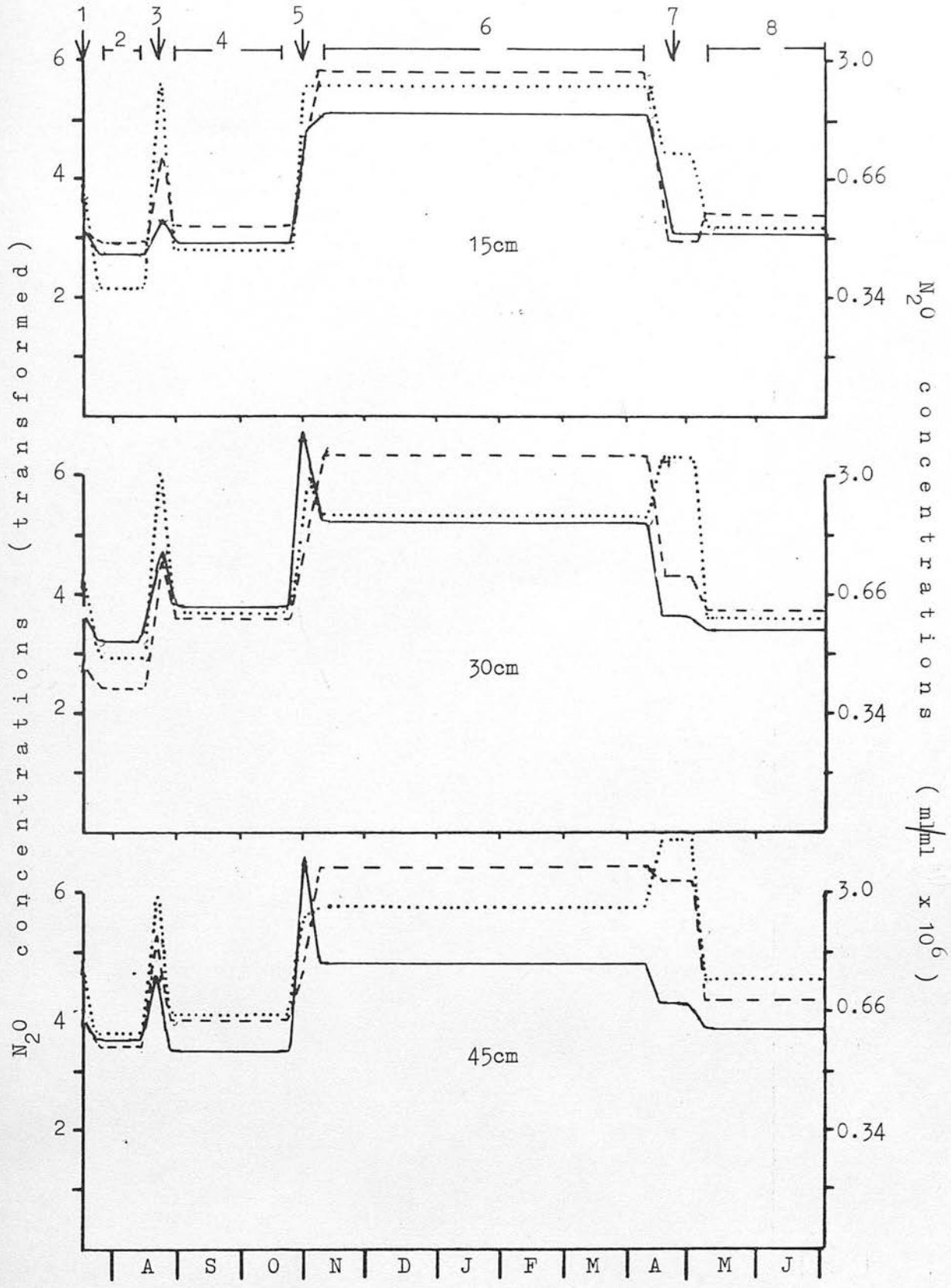


Fig. 3.25. Means of N_2O concentrations for depths and treatments during 8 periods

····· slurried --- fertilised —— control

Table 3.7 Means of transformed O_2 concentrations over 8 periods

	Mean O_2 Concentration in Period								Least sig. diff.at 0.0 level
	1	2	3	4	5	6	7	8	
Overall Mean	2.37 (20.30)	1.96 (20.56)	3.33 (19.01)	2.81 (19.86)	3.75 (17.93)	3.52 (18.58)	3.52 (18.58)	2.33 (20.33)	0.19
15cm	2.05 (20.51)	1.66 (20.70)	3.04 (19.54)	2.54 (20.15)	3.60 (18.37)	3.22 (19.23)	2.60 (20.09)	1.90 (20.59)	0.65
30cm	2.32 (20.33)	2.04 (20.52)	3.44 (18.77)	2.92 (19.71)	3.89 (17.46)	3.53 (18.56)	3.63 (18.29)	2.14 (20.46)	0.65
45cm	2.74 (19.94)	2.18 (20.43)	3.51 (18.61)	2.97 (19.64)	3.77 (17.87)	3.82 (17.70)	4.31 (15.56)	2.94 (19.69)	0.65
Control	2.25 (20.39)	2.05 (20.51)	3.18 (19.30)	2.94 (19.69)	3.72 (18.03)	3.64 (18.26)	3.38 (18.91)	2.39 (20.28)	0.65
Slurry	2.62 (20.07)	1.98 (20.55)	3.68 (18.15)	2.50 (20.19)	4.18 (16.24)	3.67 (18.17)	4.05 (16.83)	2.47 (20.21)	0.65
Fertiliser	2.25 (20.39)	1.86 (20.61)	3.12 (19.41)	2.98 (19.63)	3.36 (18.95)	3.25 (19.17)	3.12 (19.41)	2.13 (20.46)	0.65

The reverse transform of the means (ml ml^{-1} x 10^2) is given in brackets

Table 3.8 Means of transformed N_2O concentrations over 8 periods

	Mean N_2O Concentrations in Period								Least sig. diff. at 0.05 level
	1	2	3	4	5	6	7	8	
Overall	3.74 (0.57)	3.01 (0.43)	4.91 (1.20)	3.45 (0.50)	5.09 (1.38)	5.69 (2.28)	4.64 (0.99)	3.67 (0.55)	0.24
15cm	3.41 (0.49)	2.61 (0.38)	4.43 (0.86)	2.95 (0.42)	5.09 (1.38)	5.55 (2.02)	3.50 (0.51)	3.22 (0.46)	0.41
30cm	3.60 (0.54)	2.85 (0.41)	5.10 (1.40)	3.65 (0.55)	5.19 (1.50)	5.83 (2.58)	4.79 (1.10)	3.60 (0.54)	0.41
45cm	4.20 (0.74)	3.56 (0.53)	5.38 (1.75)	3.74 (0.57)	5.01 (1.30)	5.68 (2.26)	5.64 (2.19)	4.19 (0.73)	0.41
Control	3.61 (0.54)	3.16 (0.45)	4.17 (0.73)	3.34 (0.48)	4.67 (1.00)	5.07 (1.36)	3.83 (0.60)	3.41 (0.49)	0.41
Slurry	4.26 (0.77)	2.91 (0.41)	5.84 (2.61)	3.48 (0.51)	5.73 (2.37)	5.60 (2.11)	5.94 (2.85)	3.79 (0.59)	0.41
Fertiliser	3.35 (0.48)	2.95 (0.42)	4.74 (1.06)	3.52 (0.52)	4.88 (1.18)	6.40 (4.35)	4.16 (0.72)	3.80 (0.59)	0.41

N.B. The reverse transform of the means is (ml ml^{-1} x 10^6) given in brackets

differences between the fertilised and control plots.

In period 8 slurry and fertiliser increased N_2O significantly although there were no significant differences between treatments for O_2.

For the means over the whole period neither slurry nor fertiliser increased N_2O significantly and surprisingly O_2 concentrations were significantly higher in the fertilised plots than in the control plots. The difference between O_2 concentrations in slurried and control plots was not quite significant.

The analysis comparing means from the 8 periods showed that the decreases in O_2 and increases in N_2O over the winter were significant under all treatments, and that the slurry applications caused decreases in O_2 and increases in N_2O which were significantly different from O_2 and N_2O concentrations previous to the applications.

3.9. Conclusions

Logarithmic transformations of the data were again required to normalise the frequency distributions of all gases (see Section 2.8).

There was again no evidence of the soil acting as a sink for N_2O (see Sections 1.5.1.2 and 2.8).

In spite of increased replication, and the randomised block design, there were differences between plots which could not be ascribed to the applied treatments and must therefore have been due to random errors. This provides further evidence of the spatial heterogeneity of soil (Section 1.5.1.1).

As during the previous year, O_2 concentrations were highest in the summer, early autumn and late spring, and lowest during the winter and early spring. However, in the autumn of 1979, O_2 only began to decrease following heavy rain in October, since the summer was very dry. In the previous year concentrations were already low in early September when the experiment began (Section 2.5). In general O_2 concentrations decreased with depth, the difference between depths being greatest over the winter. In the spring O_2 concentrations returned to near ambient concentrations first at 15cm at the beginning of April, and at 45cm by the end of May, earlier than in the previous year.

As in 1978/79, N_2O concentrations were highest in the winter and early spring (see Section 2.6), agreeing with other workers (see Section 1.5.1.5), but there was no peak of N_2O as was noted in the previous winter (see Section 2.6), and N_2O remained at near ambient concentrations in the summer. Concentrations of N_2O in 1979 began to increase after October corresponding to the decrease in O_2 concentrations. There was no evidence in this years' data of increased N_2O concentrations in the surface soil in response to rainfall events as has been suggested in the literature (see Section 1.5.1.5).

In contrast to the previous year, the application of fertiliser and slurry caused no large immediate increases in N_2O concentrations. Summer and autumn applications of fertiliser did not increase N_2O at all, but slurry caused a small but significant increase in N_2O corresponding to a small decrease in O_2. The late autumn application of slurry immediately decreased O_2 and increased N_2O concentrations for several weeks and N_2O concentrations remained higher than in control plots for several months. In fertilised plots there were no immediate effects but later N_2O concentrations were higher than in control plots over the whole winter. During the winter the highest N_2O concentrations occurred in the fertilised plots, although the overall means for N_2O in the fertilised and slurried plots were not significantly different. The spring application of slurry increased N_2O since O_2 was lowest at this time in the slurried plots, whereas fertiliser had no effect.

Denitrification took place at all depths and there was evidence for more denitrification at 30cm in the fertilised plots than at other depths. Regression analysis showed that the relationship between N_2O and O_2 concentrations was significant for all treatments at all depths, the coefficient of variation being about 20% and 30% for the control and treated plots respectively. For any given O_2 concentration the predicted N_2O concentrations were over twice as high in the 1978/79 season as in 1979/80.

4. MEASUREMENT OF DIFFUSION COEFFICIENTS AND CALCULATION OF N_2O FLUXES

Gaseous diffusion coefficients in soil from several depths from the field site at Langhill Farm were measured at 3 water tensions. The diffusion coefficient for the surface soil at 2 soil water tensions was then used to estimate fluxes of N_2O in the winter of 1978/79 and 1979/80 using measured N_2O concentrations at the 15cm depth.

4.1. Sampling

Samples were taken from the field plot at Langhill Farm, using stainless steel sampling rings 5cm long and 3.8cm internal diameter, with a chamfered edge. The rings were driven into the soil and were then dug out. Samples from depths greater than 5cm were obtained by digging a flat-bottomed pit to the desired depth and then driving the ring into the soil at the bottom of the pit. Samples were taken from the 0-5, 5-10, 17.5 - 22.5, 27.5 - 32.5, and 37.5 - 42.5cm depths.

In the laboratory the grass on the 0 - 5cm samples was cut at soil surface level. Excess soil was carefully removed from the bottoms of all cores, which were then covered with pieces of muslin held in place with rubber bands. The cores were then thoroughly saturated by placing them in water about 3cm deep.

4.2. Tension Tables

The tension tables used were as described by Ball (1979), and consisted of a perspex tray, with drainage channels milled into the base, which was covered with glass microfibre paper with an overlying layer of silica flour, grade HPF2 (Nornef Minerals, Stoke on Trent), of size 10 - 50μm. The cores were placed directly onto the silica flour, the muslin cover aiding contact between water in the flour and the soil. Soil moisture tension was maintained by a constant head reservoir connected to the outlets to the perspex tray.

Cores from the 0 - 5 and 5 - 10cm depth were equilibrated for 2 weeks on the tension table at 2.5, 5, and 10kPa tension while cores from other depths were equilibrated at 2.5kPa only.

4.3. Measurement of Diffusion Coefficients

Diffusion coefficients were measured from the rate of diffusion of radioactive krypton - 85 (^{85}Kr) through the soil samples (Ball et al., 1981). The apparatus (Fig. 4.1) enables the measurement to be carried out on intact cores. After equilibration at the required tension, the soil core, still in its metal ring, was bolted into the apparatus between two air tight chambers and a 1.5ml sample of a mixture of ^{85}Kr and air (activity $0.7\mu Ci\,ml^{-1}$) from a gas cylinder was injected through a septum into the injection chamber. The rate of diffusion of ^{85}Kr through the soil sample to the receiving chamber depended on the diffusion coefficient.

During the decay of ^{85}Kr to rubidium - 85, β particles are emitted which are detected by the scintillation counters at either end of the apparatus. The concentration of ^{85}Kr in each chamber can therefore be calculated from the respective count rates. Counts over a short period of time were recorded at intervals until the system was at least half way to equilibrium.

The diffusion coefficient was calculated using Fick's 1st Law, even though steady state conditions did not apply, by assuming that:

(a) After the tracer gas has permeated all the air space in the sample, i.e. 1-10 minutes (Ball et al., 1981) the concentration gradient between the sample ends is linear.

(b) The number of tracer molecules in the soil sample is constant, i.e. the number of molecules leaving one face equals the number entering the other face.

(c) The concentration of tracer is uniform in a gas chamber at any one time.

Fick's first law can be written as:

$$VdC/dt = - D_A A \Delta C/L \qquad 4.1$$

Where: C is the concentration of tracer in the injection chamber (counts s^{-1})

ΔC is the difference between tracer concentration in the injection and receiving chamber (counts s^{-1})

t is time (s) (t = 0 is the time of injection)

D_A is the effective diffusion coefficient ($cm^2\ s^{-1}$)

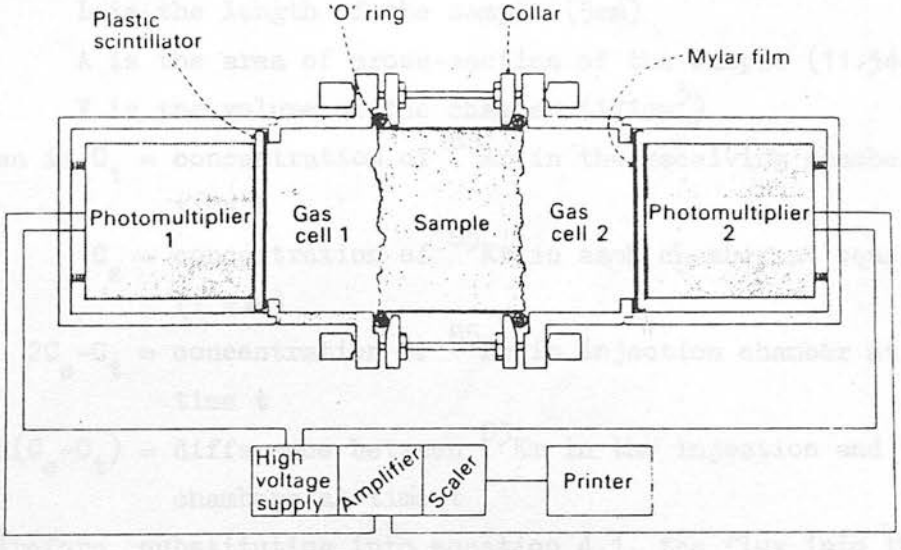


Fig. 4.1. Apparatus for measuring diffusion coefficients (from Ball, 1981)

L is the length of the sample (5cm)

A is the area of cross-section of the sample ($11.34 cm^2$)

V is the volume of the chamber ($171 cm^3$)

Then if C_t = concentration of ^{85}Kr in the receiving chamber at time t

C_e = concentration of ^{85}Kr in each chamber at equilibrium ($t = \infty$)

$2C_e - C_t$ = concentration of ^{85}Kr in injection chamber at time t

$2(C_e - C_t)$ = difference between ^{85}Kr in the injection and receiving chambers at time t

Therefore, substituting into equation 4.1, the flux into the receiving chamber is given by:

$$VdC/dt = -D_A A2(C_t - C_e)/L \qquad 4.2$$

Integrating with respect to time gives:

$$C_t = C_e\ (1 - \exp\ (-2D_A At/VL)) \qquad 4.3$$

Therefore the difference between concentrations of ^{85}Kr in the injection and receiving chambers, $2(C_e - C_t)$, is given by:

$$C_I - C_R = 2C_e \exp\ (-2D_A At/VL) \qquad 4.4$$

Where C_I is the concentration of ^{85}Kr in the injection chamber (counts s^{-1})

C_R is the concentration of ^{85}Kr in the receiving chamber (counts s^{-1})

Equation 4.4 can be rewritten as:

$$\ln\ (C_I - C_R) = \ln\ 2C_e - Kt \qquad 4.5$$

Where $K = 2D_A A/VL$

The count rate is proportional to concentration and therefore can be used to measure concentration, provided that counts are adjusted for the 'dead time' of the photomultiplier tube, the background count for the chamber, and the relative efficiency of the two chambers (Ball (1981) did not correct for dead time).

Instead of using a best fit programme, using equation 4.4, to determine C_e and D_A (Ball, 1981), the regression coefficient (-K) of $\ln\ (C_I - C_R)$ on t was determined (equation 4.5 and Fig. 4.2) and from this D_A was calculated (Ball now uses the same method). As C_I and

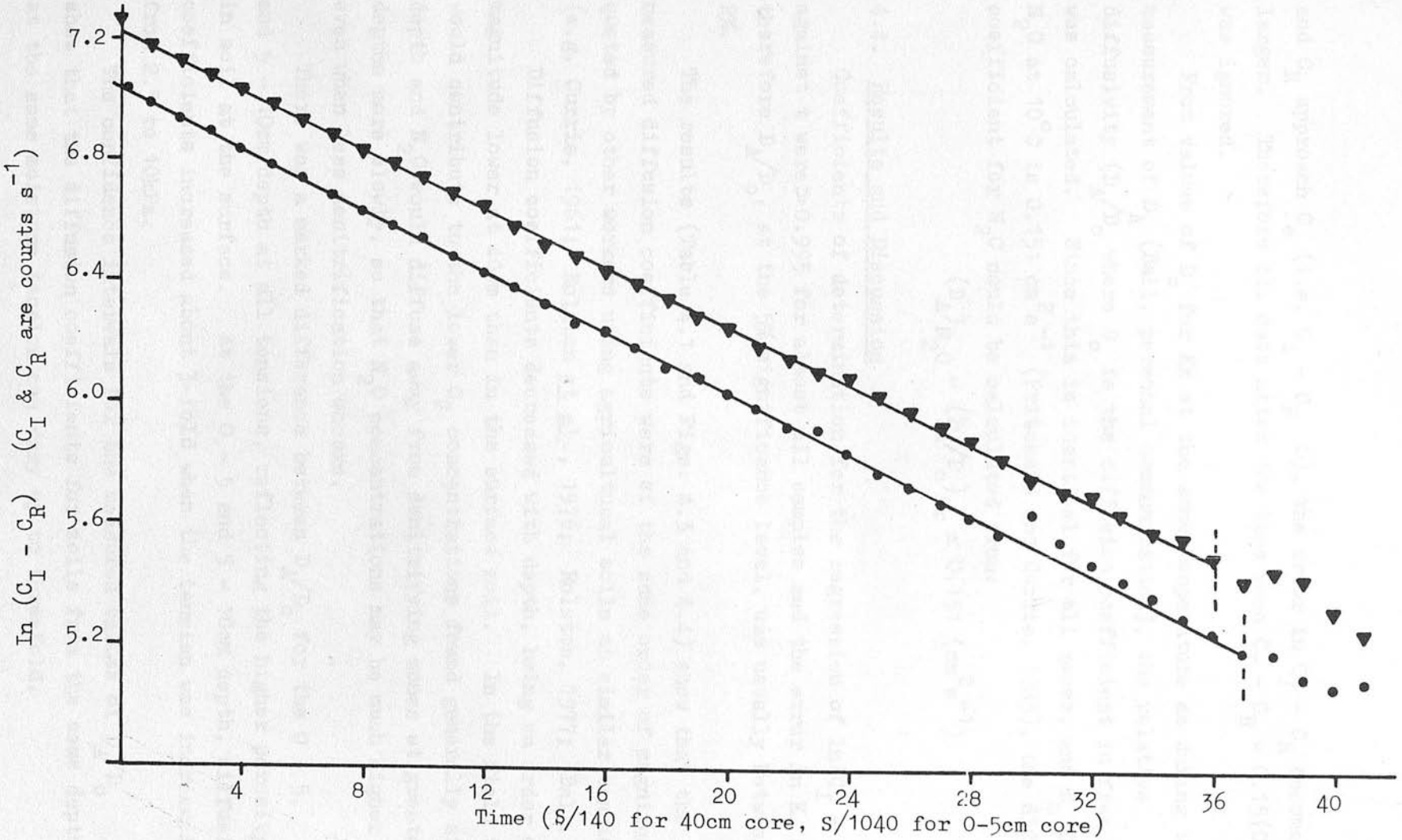


Fig. 4.2. Plot of $\ln(C_T - C_R)$ against time for 2 soil cores

40cm depth at 2.5kPa tension

0-5cm depth at 2.5kPa tension

and C_R approach C_e (i.e. $C_I - C_R$ 0), the error in $C_I - C_R$ becomes larger. Therefore all data after the time when $C_I - C_R = 0.15(C_I)_{t=0}$ was ignored.

From values of D_o for Kr at the same temperature as during the measurement of D_A (Ball, personal communication), the relative diffusivity (D_A/D_o where D_o is the diffusion coefficient in free air) was calculated. Since this is identical for all gases, and D_o for N_2O at 10°C is 0.151 cm^2s^{-1} (Pritchard and Currie, 1983), the diffusion coefficient for N_2O could be calculated from:

$$(D_A)_{N_2O} = (D_A/D_o)_{Kr} \times 0.151 \ (cm^2s^{-1}) \qquad 4.6$$

4.4. Results and Discussion

Coefficients of determination for the regression of $\ln(C_I - C_R)$ against t were >0.995 for almost all samples and the error in K, and therefore D_A/D_o, at the 5% significance level, was usually between 1 and 2%.

The results (Table 4.1 and Figs. 4.3 and 4.4) show that the measured diffusion coefficients were of the same order of magnitude as quoted by other workers using agricultural soils at similar tension (e.g. Currie, 1961; Rolston et al., 1976; Rolston, 1977; Ball, 1979).

Diffusion coefficients decreased with depth, being an order of magnitude lower at 40cm than in the surface soil. In the field this would contribute to the lower O_2 concentrations found generally at depth and N_2O would diffuse away from denitrifying zones at greater depths more slowly, so that N_2O concentrations may be much higher even when less denitrification occurs.

There was a marked difference between D_A/D_o for the 0 - 5, and 5 - 10cm depth at all tensions, reflecting the higher porosity in soil at the surface. At the 0 - 5 and 5 - 10cm depth, diffusion coefficients increased about 3-fold when the tension was increased from 2.5 to 10kPa.

The confidence intervals for the measured values of D_A/D_o show that the diffusion coefficients for soils from the same depth at the same moisture tension can vary up to threefold.

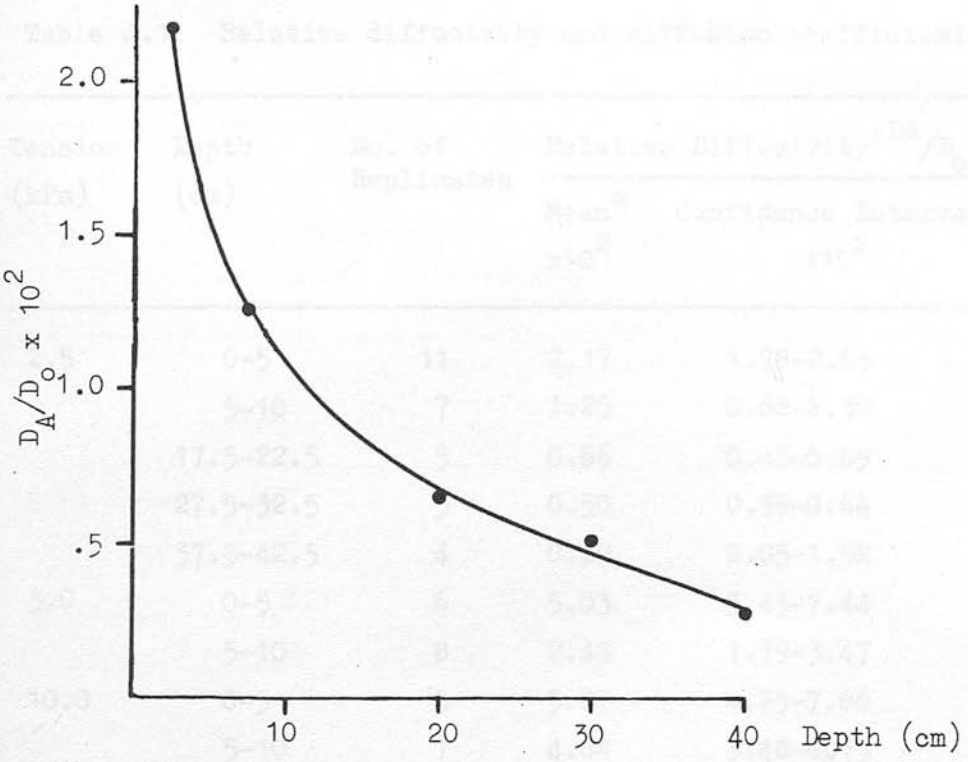


Fig. 4.3. Variation of diffusion coefficient with depth

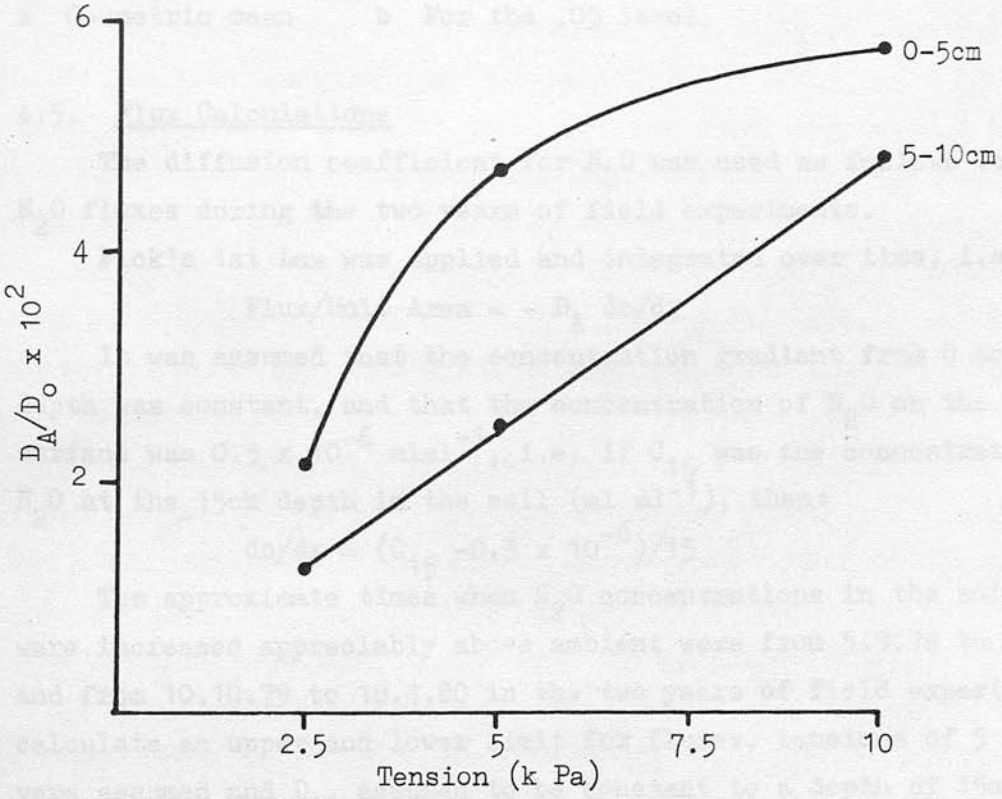


Fig. 4.4. Variation of diffusion coefficient with soil moisture tension

Table 4.1. Relative diffusivity and diffusion coefficients for N_2O

Tension (kPa)	Depth (cm)	No. of Replicates	Relative Diffusivity (D_A/D_o)		Diffusion Coefficient for N_2O
			Mean[a] $x10^2$	Confidence Interval[b] $X10^2$	$(cm^2 s^{-1} x10^3)$
2.5	0-5	11	2.17	1.78-2.65	3.28
	5-10	7	1.25	0.68-2.30	1.89
	17.5-22.5	5	0.66	0.48-0.89	1.00
	27.5-32.5	5	0.50	0.39-0.64	0.76
	37.5-42.5	4	0.29	0.05-1.58	0.44
5.0	0-5	6	5.03	3.43-7.44	7.60
	5-10	8	2.49	1.79-3.47	3.76
10.0	0-5	5	5.87	4.25-7.86	8.86
	5-10	7	4.84	3.48-6.73	7.31

a Geometric mean b For the .05 level.

4.5. Flux Calculations

The diffusion coefficient for N_2O was used as follows to estimate N_2O fluxes during the two years of field experiments.

Fick's 1st Law was applied and integrated over time, i.e.:

$$\text{Flux/Unit Area} = - D_A \, dc/dx \qquad 4.7$$

It was assumed that the concentration gradient from 0 to 15cm depth was constant, and that the concentration of N_2O on the soil surface was 0.3×10^{-6} $mlml^{-1}$, i.e. if C_{15} was the concentration of N_2O at the 15cm depth in the soil ($ml\ ml^{-1}$), then:

$$dc/dx = (C_{15} - 0.3 \times 10^{-6})/15 \qquad 4.8$$

The approximate times when N_2O concentrations in the soil profile were increased appreciably above ambient were from 5.9.78 to 13.6.79 and from 10.10.79 to 18.4.80 in the two years of field experiments. To calculate an upper and lower limit for fluxes, tensions of 5 and 1kPa were assumed and D_A, assumed to be constant to a depth of 15cm, was taken as that for the 5-10cm depth from Table 4.1 for 5kPa, and by extrapolation of the 5-10cm depth line of Fig. 4.4 for 1kPa.
The flux was calculated by substituting

equation 4.8 into 4.1 and converting the flux from ml $cm^{-2}s^{-1}$ to kg N ha^{-1} d^{-1}. It was assumed that there was no flux from Jan. 25th to Feb. 16th 1979 because the ground was frozen.

Table 4.2 Estimated losses of N_2O - N in winter 78/79 and 79/80 under 3 treatments

		Total Loss of N_2O-N (kg ha^{-1})		
		Control	Slurried	Fertilised
78/79	Upper limit[a]	5.7	5.3	11.2
	Lower limit[b]	1.3	1.3	2.7
79/80	Upper limit[a]	0.8	1.9	2.0
	Lower limit[b]	0.2	0.4	0.5

Notes
a assuming a tension of 5kPa
b assuming a tension of 1kPa

In the preliminary field experiment (Fig. 4.5) it appeared that most of the loss occurred immediately following the thaw. This is probably unrealistic since at this time the soil, even at the 15cm depth, was close to saturation, and therefore the flux was over-estimated. If a tension of 1kPa is assumed for the first sampling occasion after the thaw, and 5kPa at all other times, the estimated losses of 6.0, 3.1 and 2.7 kg N ha^{-1} in the fertilised, slurried, and control plot, are probably more realistic than the upper limit shown in Table 4.2.

In the two weeks following the application of slurry and fertiliser, between 0.2 and 1 kg N ha^{-1} was lost (assuming a moisture tension of 1 and 5 kPa respectively), while at other times fluxes of N_2O were low in all plots. Thus in spite of the large slurry application, losses of N_2O-N were much higher from the fertilised plot.

In the randomised block experiment (Fig. 4.6), estimated fluxes were much lower than in the previous year. The flux of N_2O from control plots was less than half that from the treated plots. Fluxes were highest in the autumn in the slurried plot and over the winter from the fertilised plot.

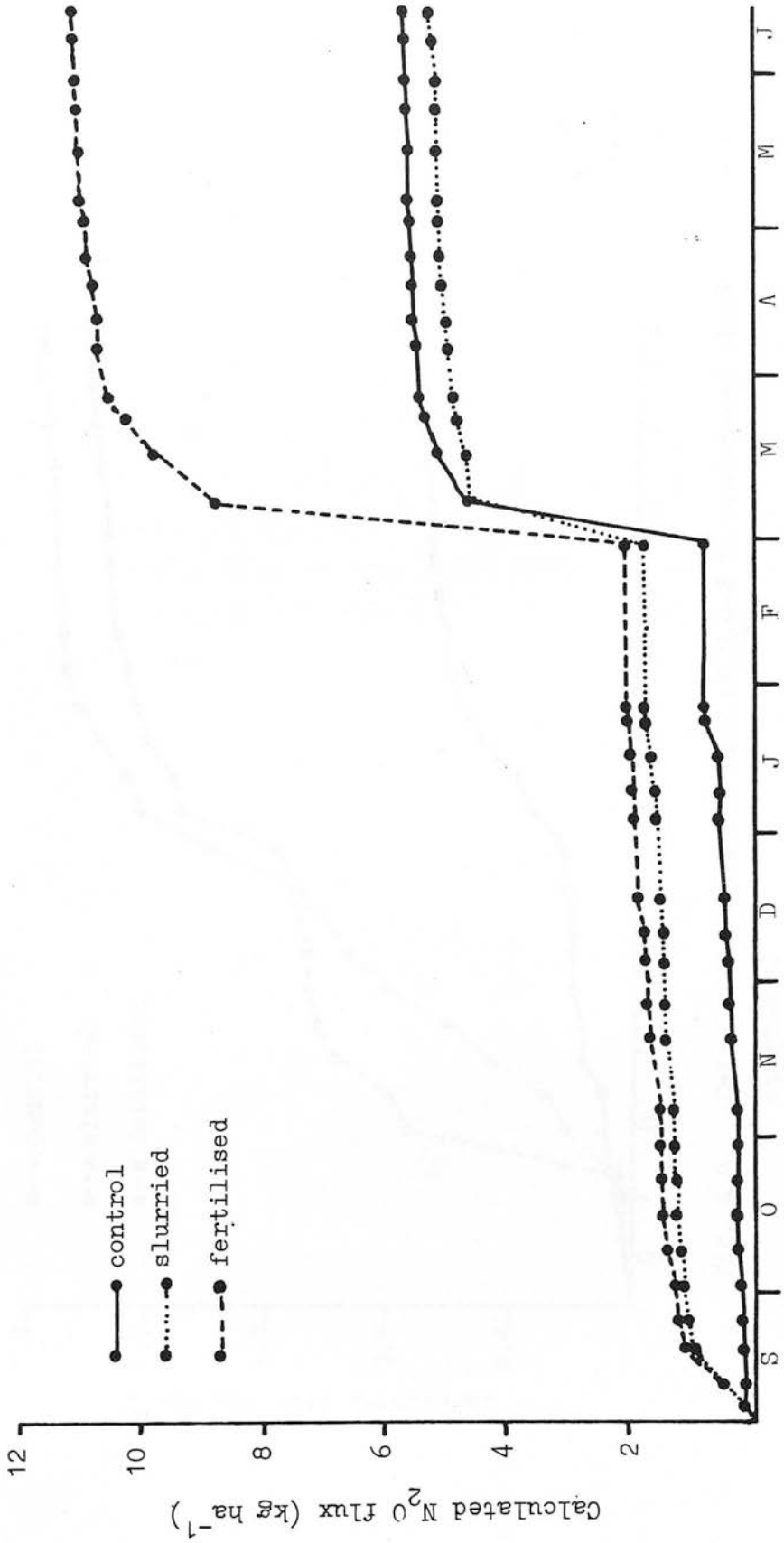


Fig. 4.5. Calculated cumulative flux of N_2O-N in preliminary field experiment

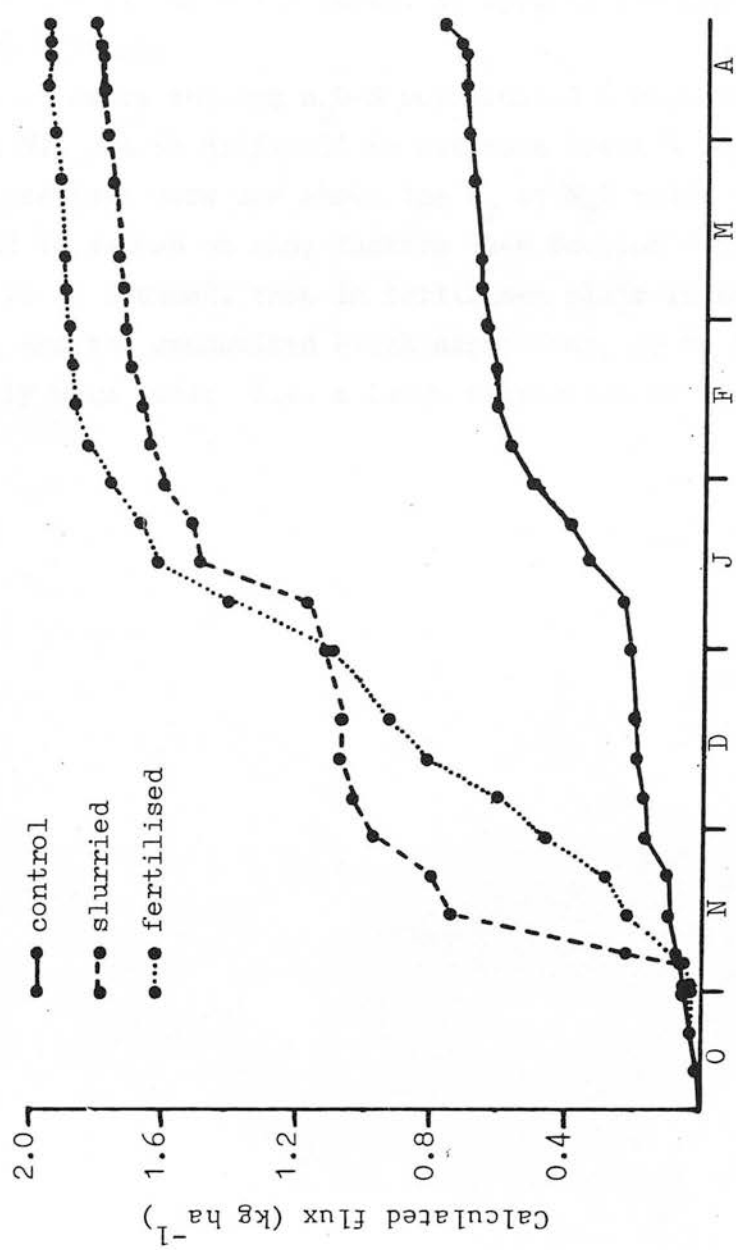


Fig. 4.6. Calculated cumulative flux of N_2O-N in randomised block experiment

Although the assumptions made were not strictly valid, the estimated flux was probably between the upper and lower limits given in Table 4.2. Since soils are usually at lower tensions than field capacity during the winter, the lower estimate was probably more accurate than the higher.

Estimated fluxes ranged from 2.3×10^{-4} to 5.3×10^{-1} kg N ha^{-1} d^{-1}, which as Table 1.5 shows, is typical for non-irrigated agricultural systems.

Total N losses through N_2O-N represented a significant percentage of applied N. It is difficult to estimate total N loss from the results; previous work has shown the N_2 to N_2O ratio to vary from 2 to 37 and to depend on many factors (see Section 1.5.3). If a factor of 10 is assumed, then in fertilised plots in the preliminary experiment and the randomised block experiment, up to 60 and 20kg N ha^{-1} respectively were lost; i.e. a large proportion of the N applied.

5. INCUBATION EXPERIMENTS TO DETERMINE RATES OF DENITRIFICATION

The aim of the incubation experiments was to investigate the kinetics of denitrification and the relative rates of reduction of NO_3^- and N_2O, and to determine under what conditions high N_2O concentrations are likely during denitrification. Secondary aims were to see how effectively C_2H_2 inhibited N_2O reduction and whether rates of NO_3^- and N_2O reduction differed between soil which had recently received large amounts of slurry and unamended soil.

5.1 Soil

The soil was from the field site described in Section 2.1, sampled on 25th September 1978 from the control and slurried plots. The slurried plot had received a large application of slurry a few weeks previously. Samples from 0-10, 10-20, 20-30 and 30-40cm depths were air-dried, passed through a 2mm sieve and stored in the laboratory. Nitrate analysis was carried out as described in Appendix 6 on all soils (Table 5.1).

Table 5.1. Nitrate concentrations in soils used for incubation experiment.

Depth (cm)	Nitrate(μg-N g^{-1} soil)	
	Slurried Plot	Control Plot
0 - 10	111	25
10 - 20	48	10
20 - 30	35	7
30 - 40	3.7	0.7

5.2 Incubation Procedure

Three grams of soil were covered with 10ml of water in glass tubes (internal volume 26.4ml) and sealed with Subaseal rubber stoppers. Air was flushed out by a stream of N_2 or C_2H_2 entering and leaving the tube via hypodermic needles through the stopper for 1h. (Fig. 5.1). Concentrations of O_2 in the glass tubes during the experiments ranged from 0 to 0.03ml ml^{-1} but the differences between replicates were not related to O_2 concentrations, i.e. the layer of water and low gaseous O_2 concentrations were sufficient to maintain anaerobic conditions in the soil.

The incubations were carried out in the laboratory at 23°C. Before sampling with a syringe previously flushed with N_2, the tubes were shaken to establish equilibrium between the liquid and gaseous phases. After sampling an equal volume of N_2 was injected into the tube to maintain atmospheric pressure. Analysis for N_2O was as described in Appendix 3.B.

5.3 Incubation Experiments

Experiment 1. Measurement of rates of NO_3^- reduction.

If C_2H_2 completely inhibits N_2O reduction without affecting NO_3^- reduction, then the rate of N_2O formation should equal the rate of NO_3^- reduction. At high enough C_2H_2 concentrations previous work has shown this hypothesis to be valid (see Section 1.2.4.1).

Soil samples from each depth and from both plots were incubated in triplicate for 12 days as described in Section 5.2, C_2H_2 being used to flush out the tubes. At the end of the incubation, gas in the tubes was analysed for CO_2 to compare respiration rates. The tubes were then reflushed with C_2H_2 to remove all N_2O and reincubated to see whether high N_2O concentrations were inhibiting further NO_3^- reduction. The soils were then analysed for NO_3^- as described in Appendix 6.

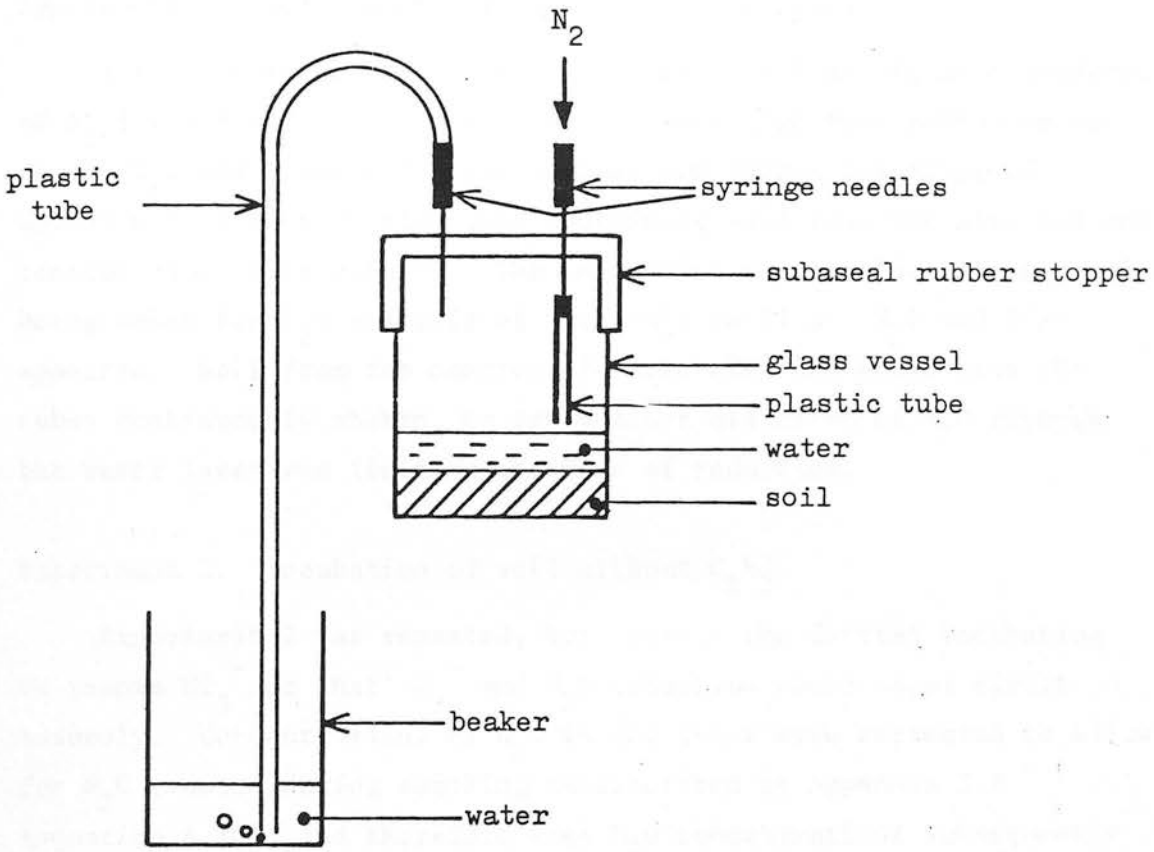


Fig. 5.1. Method for flushing tubes with N_2

Experiment 2. Measurement of rates of N_2O reduction.

Soil samples were first incubated in triplicate in an atmosphere of N_2 for 4 days to remove all NO_3^-. Tubes were then reflushed to remove N_2O and 0.2ml and 0.1ml of pure N_2O (250µg and 125µg of N_2O-N) were injected into tubes containing soil from the slurried and control plots respectively. The incubation was continued, gas samples being taken for N_2O analysis at intervals until all N_2O had disappeared. Soil from the control plot was also incubated with the tubes continuously shaken, to see whether diffusion of N_2O through the water layer was limiting the rate of reduction.

Experiment 3. Incubation of soil without C_2H_2.

Experiment 2 was repeated, but without the initial incubation to remove NO_3^-, so that NO_3^- and N_2O reduction could occur simultaneously. Concentrations of N_2O in the tubes were corrected to allow for N_2O removed during sampling as described in Appendix 3.B (equation A.10), and therefore when N_2O concentrations subsequently decreased it was necessary to multiply measured N_2O concentrations by a factor F where:

F = corrected maximum concentration/measured maximum concentration 5.1

5.4 Results and Discussion

Experiment 1.

There was a lag period of 1 day for soil from the 30-40cm depth before denitrification began, but not at other depths (Figs. 5.2 and 5.3). The rate of N_2O formation increased to a maximum after about 10h and 20h in soil from the control and slurried plots respectively, but during the first 10h N_2O concentrations were similar for soils from the same depth from both plots. During the time taken to reach maximum rates either NO_3^- reductase was being synthesised or the denitrifying population built up, and the maximum rate of reduction

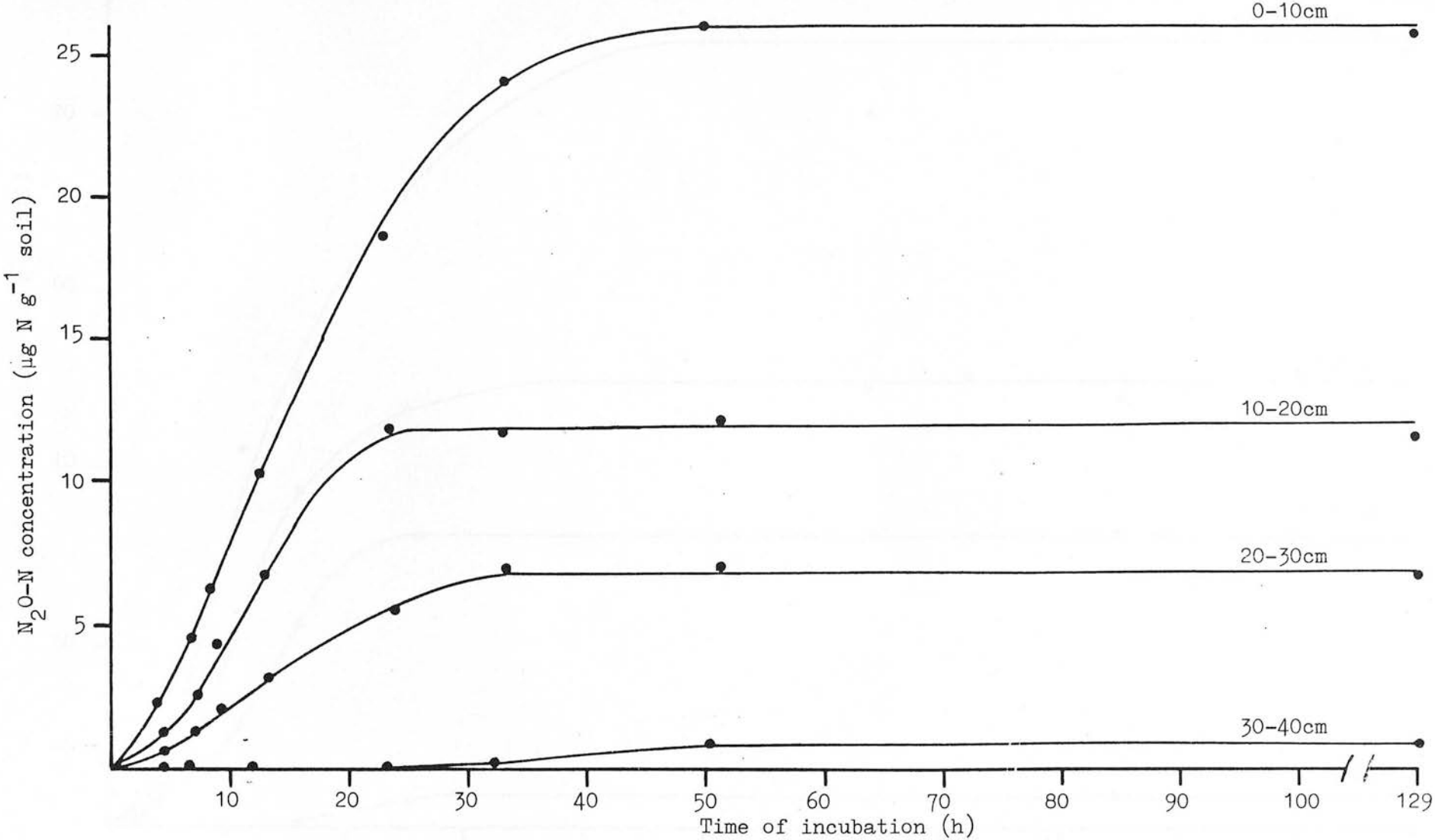


Fig. 5.2. Formation of N_2O in an incubation of soil from the control plot with C_2H_2

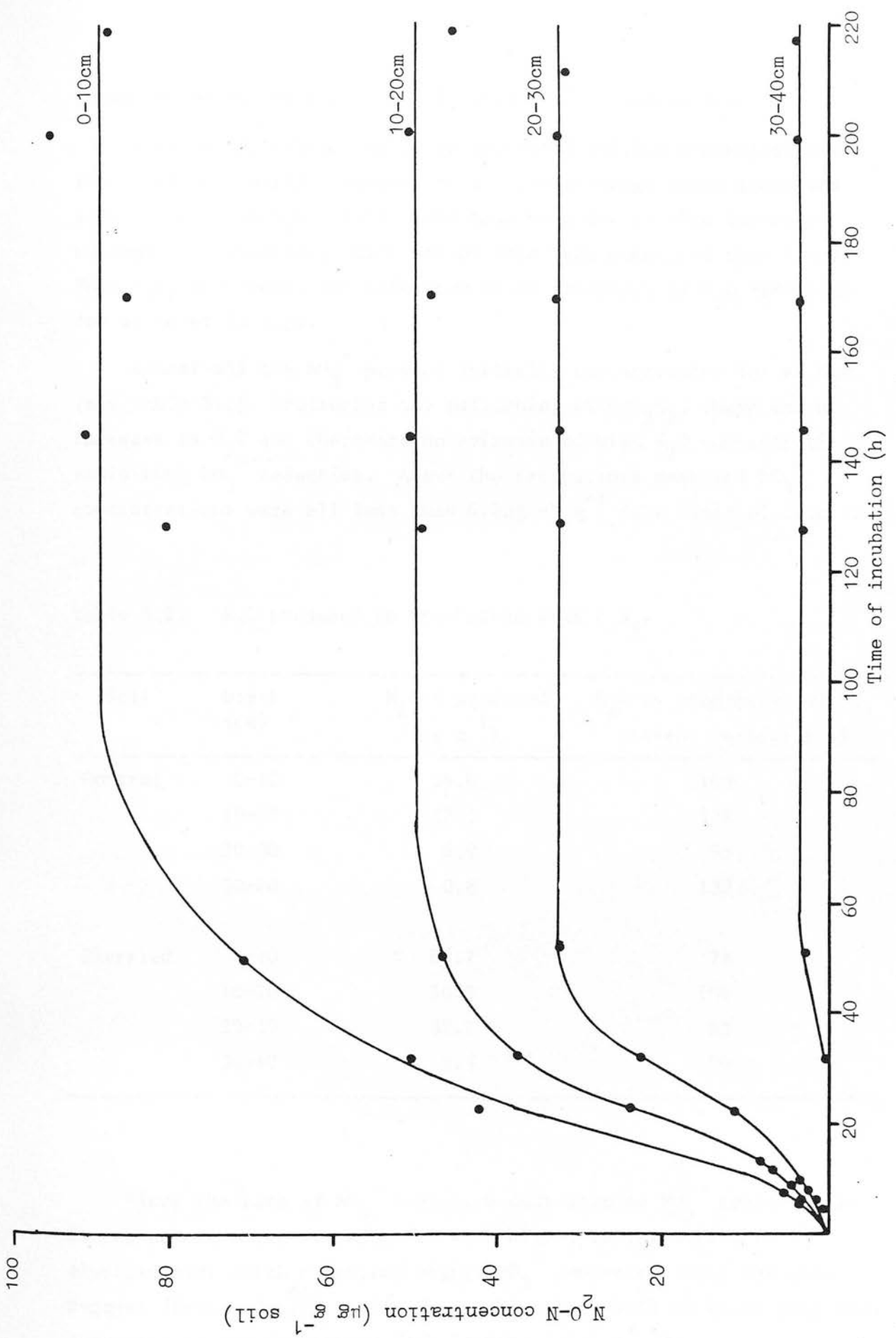


Fig. 5.3. Formation of N_2O in an incubation of soil from the slurried plot with C_2H_2

seemed to be determined by the initial NO_3^- concentration.

Rates of N_2O formation later decreased and N_2O concentrations then remained fairly constant for 10 days although later there was some decrease in N_2O. This could have been due to slow leakages through the subaseals, which had by then been punctured many times. Thus C_2H_2 was completely effective as an inhibitor of N_2O reduction for at least 10 days.

Almost all the NO_3^- present initially was accounted for as N_2O (see Table 5.2). Following the reflushing with C_2H_2, there was no increase in N_2O and therefore no evidence of high N_2O concentrations inhibiting NO_3^- reduction. After the incubations measured NO_3^- concentrations were all less than 0.2μg -N g^{-1} (the limit of detection).

Table 5.2. N_2O produced in incubation with C_2H_2.

Soil	Depth (cm)	N_2O-N produced (μg g^{-1})	N_2O as proportion of NO_3^- present initially (%)
Control	0-10	26.0	103
	10-20	12.1	118
	20-30	6.9	95
	30-40	0.8	132
Slurried	0-10	88.7	78
	10-20	50.0	104
	20-30	32.7	93
	30-40	3.3	90

Since the rate of NO_3^- reduction decreased as NO_3^- concentrations decreased and rates of reduction were higher in the soil from the slurried plot which contained higher NO_3^- concentrations, the data suggest first order or Michaelis-Menten kinetics, both of which have been suggested in the literature (see Section 1.4.3.).

Although there were few points in Figures 5.2 and 5.3 after maximum rates had been reached, tests were made to see whether the data after this time fitted the Michaelis-Menten equation (see Equation 1.17) or first order kinetics (Equation 5.2 below).

$$[NO_3^- - N] = [NO_3 - N]_{t=0} \exp(-kt) \qquad 5.2$$

where k is the 1st order rate constant (h^{-1})

t is time (h)

$[NO_3^- - N]$ is the nitrate concentration ($\mu g\ N\ g^{-1}$)

To test for Michaelis-Menten kinetics $[NO_3^- - N]/v$ was plotted against $[NO_3^- - N]$, but this did not give a straight line for any of the soils incubated. To test for 1st order kinetics $\ln [NO_3 - N]/[NO_3 - N]_{t=0}$ was plotted against t. This gave a straight line for some of the data for soil from the control plot from the 0-10cm depth and from the slurried plot from the 0-10, and 10-20cm depth.

Maximum rates of NO_3^- reduction (Table 5.3) were of the same order of magnitude as other rates for cultivated soils published in the literature (see Section 1.4.3) and decreased with depth, especially below 30cm as was also found by Germon and Couton (1981). Higher rates in soil from the slurried plot may have been due to higher nitrate concentrations or to increased microbial activity as a result of the readily oxidisable material added as slurry. Respiration was also increased in soil from the slurried plot (Table 5.3).

Table 5.3. Maximum rates of N_2O formation and final CO_2 concentrations in incubation with C_2H_2.

Depth (cm)	Maximum rates of N_2O formation ($\mu g - N\ g^{-1}\ h^{-1}$)		Final CO_2 concentration ($ml\ ml^{-1}$)	
	Slurried	Control	Slurried	Control
0-10	2.56	1.00	0.051	0.033
10-20	1.96	0.74	0.033	0.024
20-30	1.28	0.36	0.027	0.015
30-40	0.14	0.04	0.010	0.006

Experiment 2.

The needle used for injecting N_2O was found to be partially blocked, resulting in some variation in initial N_2O concentrations in the tubes. To enable the data to be compared more easily, zero time in Figures 5.4 and 5.5 was taken as the time when the amount of N_2O-N in the tubes was 100μg (4.05×10^{-3} ml ml^{-1}) and 220μg (8.9×10^{-3} ml ml^{-1}) for soil from the control and slurried plot respectively.

Rates of N_2O reduction decreased as N_2O concentrations decreased in all samples, suggesting Michaelis-Menten or 1st order kinetics. However, a plot of $[N_2O\text{-}N]/v$ against $[N_2O\text{-}N]$ (see Equation 1.17) did not give a straight line for any of the soils, indicating that the reduction did not follow Michaelis-Menten kinetics. A plot of $\ln([N_2O\text{-}N]/[N_2O\text{-}N]_{t=0})$ against time (Fig. 5.6) gave a straight line until N_2O concentrations reached about 0.2 of the initial concentration but at lower concentrations the rate was higher than would be predicted from a first order reaction.

First order rate constants for initial parts of the graphs and the rates of reduction at 30 and 10μg N_2O-N g^{-1} are given in Table 5.4.

Table 5.4. First Order rate constants and rates at 2 concentrations for N_2O reduction.

Depth (cm)	1st order rate constant (h^{-1})		Rate of N_2O-N reduction (μg $g^{-1}h^{-1}$) at N_2O-N concentration of			
			30μg g^{-1}		10μg g^{-1}	
	Slurried	Control	Slurried	Control	Slurried	Control
0-10	0.093	0.122	2.8	4.4	1.3	0.9
10-20	0.071	0.095	2.1	3.3	1.1	1.0
20-30	0.061	0.110	1.9	4.4	0.9	1.1
30-40	0.035	0.043	1.4	1.0	1.1	1.1

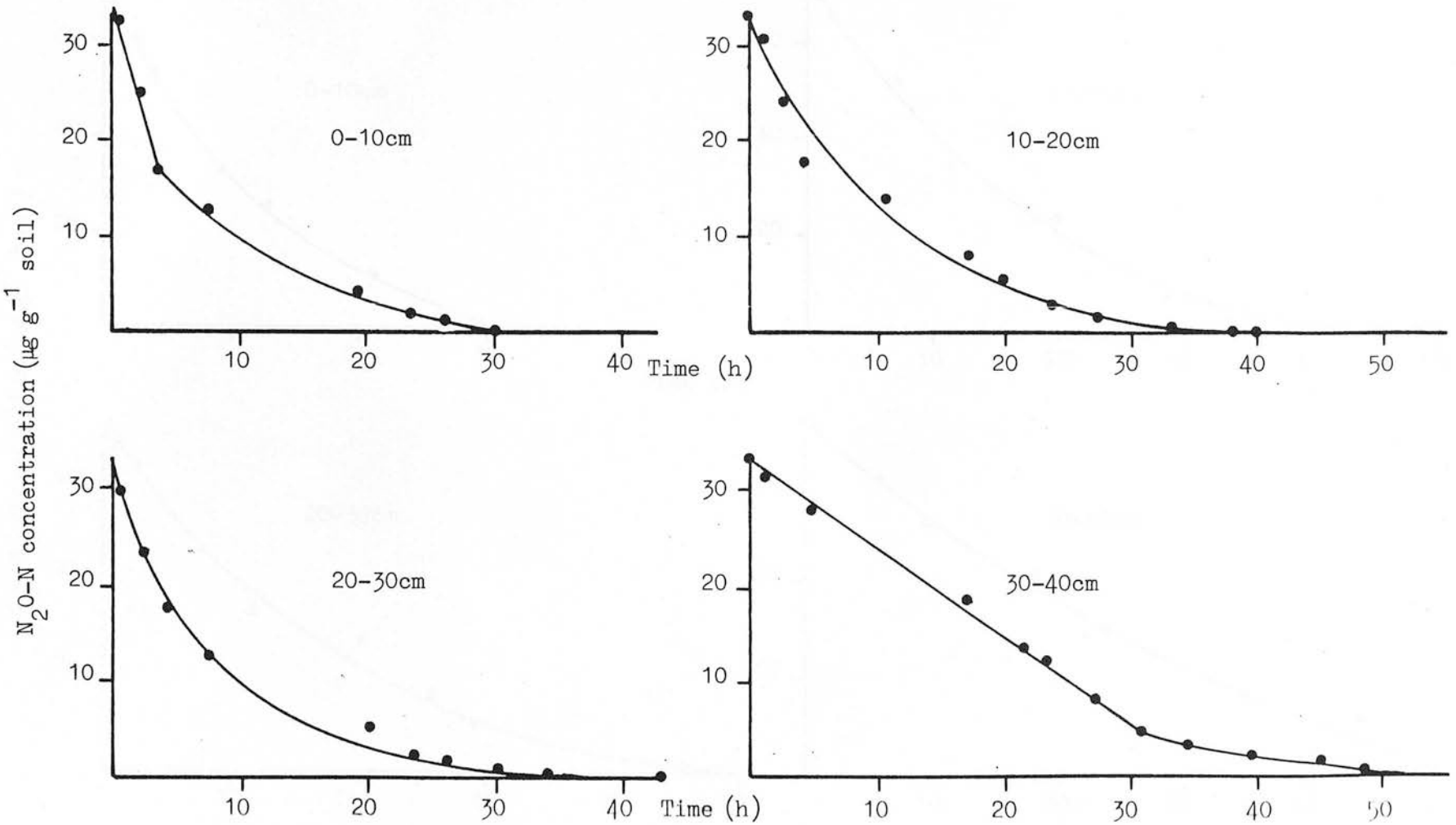


Fig. 5.4. N_2O reduction by soil from the control plots

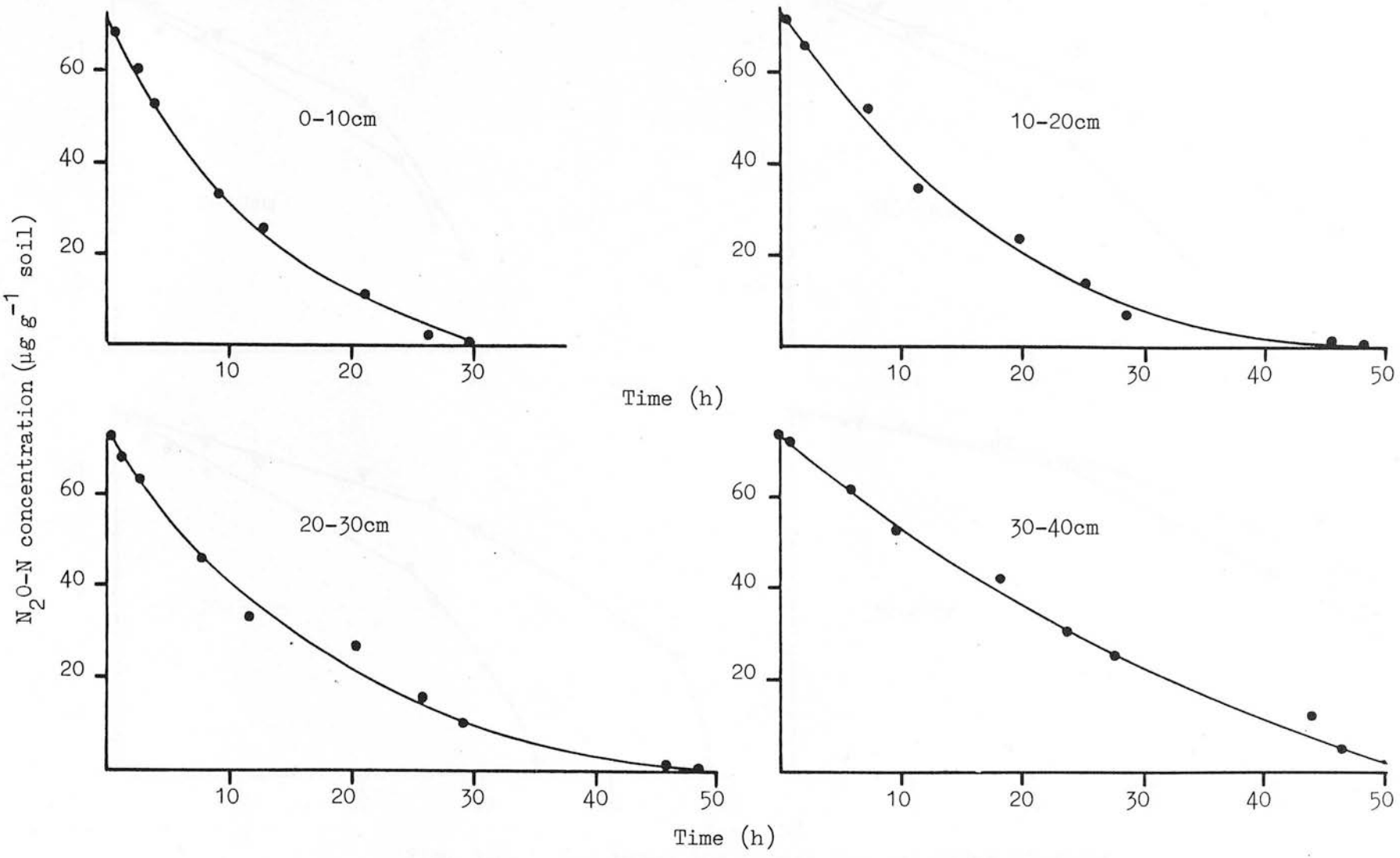


Fig. 5.5. N_2O reduction by soil from the control plots

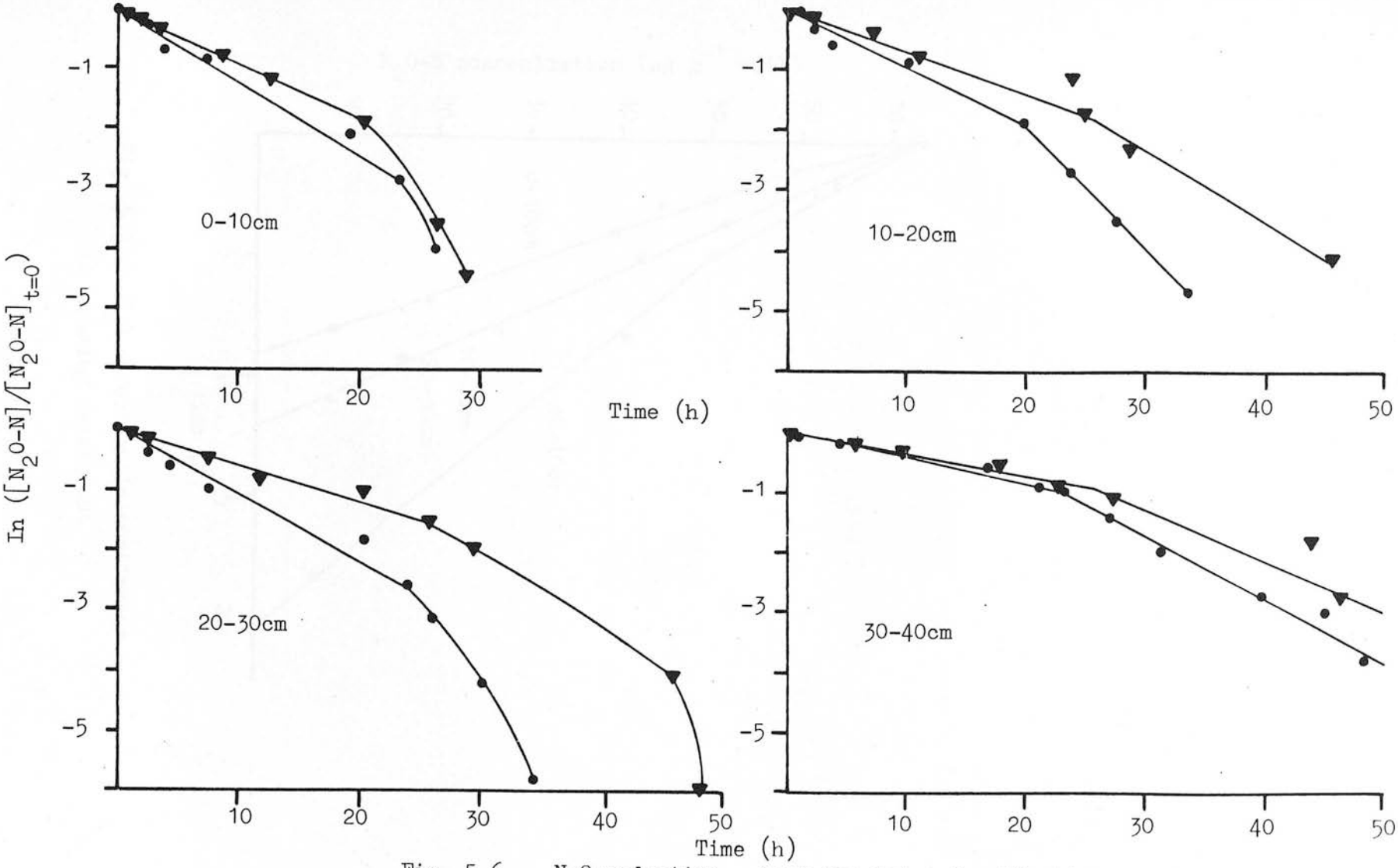


Fig. 5.6. N_2O reduction - test for 1st order kinetics

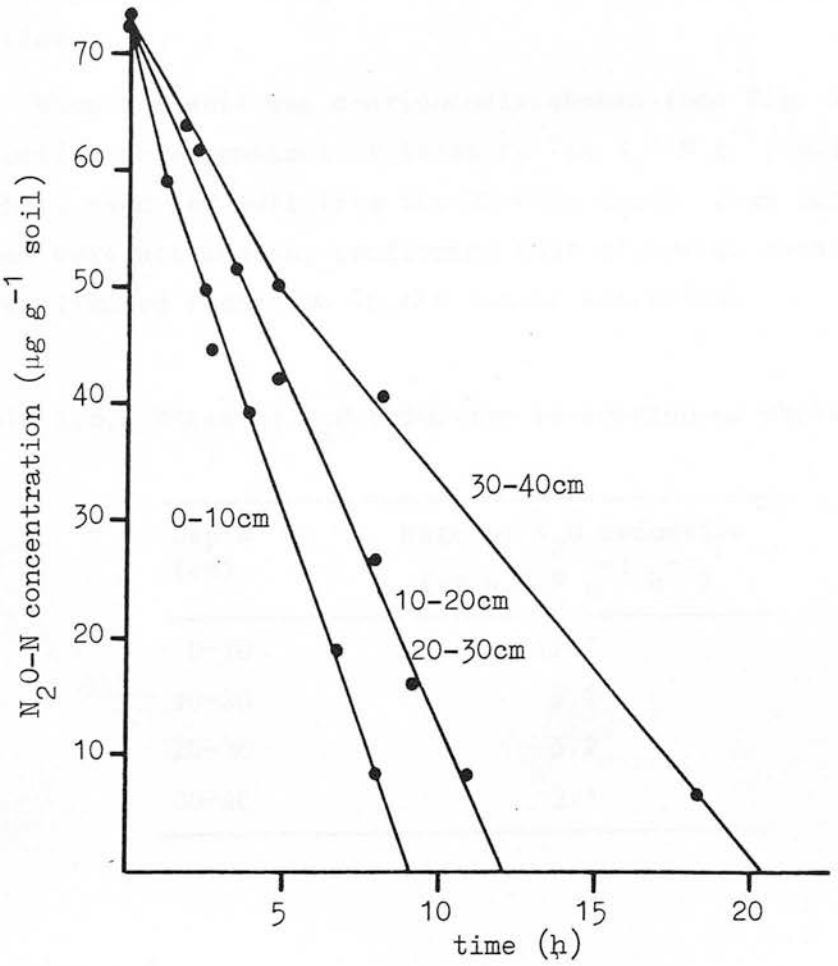


Fig. 5.7. N_2O reduction in continuous shaking experiment

It is clear from the Table 5.4 that for soils from all depths, at high N_2O concentrations, rates of N_2O reduction were much higher than for NO_3^- reduction at high nitrate concentrations (c.f. Table 5.3). The rates of reduction in Table 5.4 are higher than those in the published literature (see Table 1.2) but this may be because of the high N_2O concentrations used in the experiment. At lower N_2O concentrations rates are comparable with those for NO_3^- reduction for the surface soil but much higher for soil from lower depths. Since rates of N_2O reduction were more or less the same for soil from both plots at all depths, diffusion through the water layer was probably limiting reduction.

When the soil was continuously shaken (see Fig. 5.7) rates of reduction were constant at least to 7μg N_2O-N g^{-1}, and were much higher, even for soil from the 30-40cm depth, than rates when the tubes were not shaken, confirming that diffusion through the water layer limited reduction in the latter situation.

Table 5.5. Rates of N_2O reduction in continuous shaking experiment.

Depth (cm)	Rate of N_2O reduction (μg N_2O-N g^{-1} h^{-1})
0-10	12.7
10-20	5.2
20-30	5.2
30-40	3.1

Experiment 3.

In the incubation with no C_2H_2 or N_2O added, N_2O concentrations increased to a maximum and then declined to zero (Figs. 5.8 and 5.9). At all depths, but especially at 0-10cm and 10-20cm, N_2O concentrations for soil from the slurried plot were very much higher than for soil from the control plot. The peak N_2O concentrations decreased sharply with depth, and for soil from 30-40cm depth, did not exceed

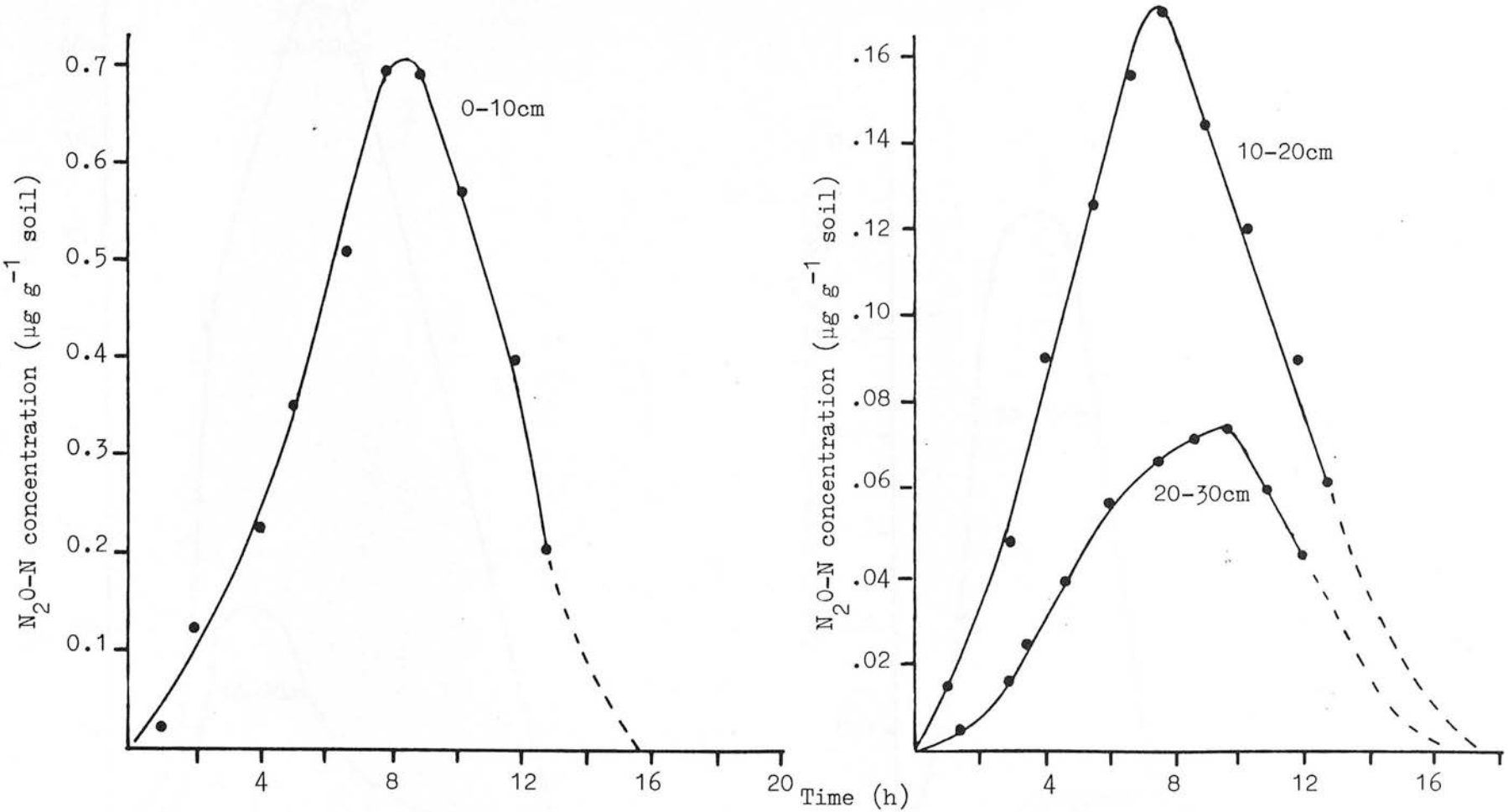


Fig. 5.8. N_2O concentrations in incubated soil from the control plot

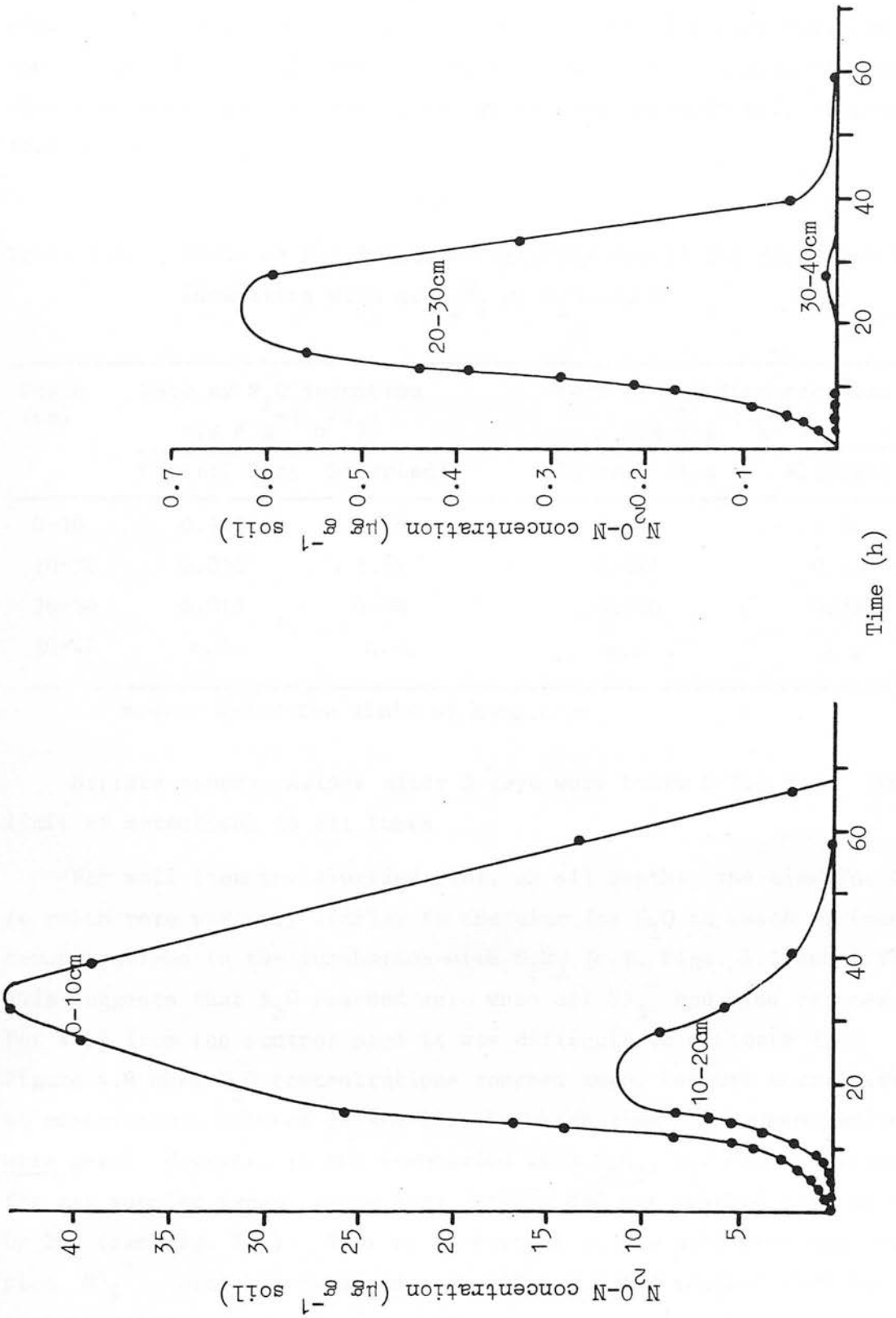


Fig. 5.9. N_2O concentrations in soil from the slurried plot

1×10^{-6} ml ml^{-1} and 3×10^{-6}ml ml^{-1} for the control and slurried plot respectively. Initially rates of N_2O formation were low, and then remained constant over some hours (Table 5.6). During the period when N_2O concentrations decreased, rates were approximately constant (Table 5.6).

Table 5.6. Rates of N_2O formation (maximum rates) and reduction in incubation with no C_2H_2 or N_2O added.

Depth (cm)	Rate of N_2O formation ($\mu g\ N\ g^{-1}\ h^{-1}$)		Rate of N_2O disappearance ($\mu g\ N\ g^{-1}\ h^{-1}$)	
	Control Plot	Slurried	Control Plot	Slurried
0-10	0.127	4.70	0.143	1.34
10-20	0.033	1.54	0.024	0.757
20-30	0.015	0.08	0.016	0.053
30-40	n.d.	n.d.	n.d.	n.d.

n.d. - below the limit of detection

Nitrate concentrations after 3 days were below 0.2$\mu g\ N\ g^{-1}$ (the limit of detection) in all tubes.

For soil from the slurried plot, at all depths, the time for N_2O to reach zero was very similar to the time for N_2O to reach maximum concentrations in the incubation with C_2H_2 (c.f. Figs. 5.3 and 5.9). This suggests that N_2O reached zero when all NO_3^- had been reduced. For soil from the control plot it was difficult to estimate from Figure 4.8 when N_2O concentrations reached zero, because there were no measurements between 12 and 28h, by which time N_2O concentrations were zero. However, in the incubation with C_2H_2, N_2O concentrations for all samples except those from 10-20cm had not reached the maximum by 28h (see Fig. 5.2). Thus in experiment 3, for soil from the control plot NO_3^- probably remained even after N_2O had reached very low concentrations.

For soil from the slurried plot from 0-10cm and 10-20cm, N_2O

concentrations were very similar in the incubations with and without C_2H_2 for the first 15h (c.f. Figs. 5.3 and 5.9) suggesting that the high NO_3^- concentrations inhibited N_2O reduction in Experiment 3, as has been suggested in the literature (see Section 1.4.4.7). Inhibition was total until NO_3^- concentrations reached 86µg N g^{-1} in the soil from 0-10cm, and 40µg N g^{-1} in that from 10-20cm. After 15h rates of N_2O formation were lower than in the incubation with C_2H_2, implying that N_2O was also being reduced. At other depths and for soil from the control plot, even initially N_2O concentrations were lower than in the incubation with C_2H_2, probably because NO_3^- concentrations were not high enough to inhibit N_2O reduction.

Rates of N_2O disappearance were also much lower than rates measured in the incubation with N_2O (Table 5.4) except for soil from the slurried plot at 0-10cm and 10-20cm, probably because N_2O concentrations were much lower than those of Experiment 2.

Although in Experiments 1, and 2, NO_3^- and N_2O reduction was not a straightforward 1st order reaction, there was clear evidence in both experiments that rates were concentration-dependent. Thus initially, with high NO_3^- and low N_2O concentrations, N_2O was formed faster than it was reduced and concentrations therefore increased. Later, as N_2O concentration increased and NO_3^- concentrations decreased, the rate of production of N_2O decreased and the rate of reduction increased, leading eventually to a maximum and then a decrease in N_2O concentration. If first order kinetics is assumed then at the maximum

$$K_2/K_1 = [NO_3^- - N]/[N_2O - N] \qquad 5.3$$

where K_1 is the first order rate constant for nitrate reduction (h^{-1})

K_2 is the first order rate constant for N_2O reduction (h^{-1})

Using Figures 5.2 and 5.3 to determine NO_3^- concentrations at the time of the N_2O maximum, values of K_2/K_1 were calculated (see Table 5.7). This confirmed that rates of N_2O reduction were much

higher than for NO_3^- reduction, especially for soil from greater depths; however, at high NO_3^- concentrations, i.e. in soil from the slurried plot from the 0-10cm and 10-20cm depth, rates of NO_3^- and N_2O reduction were comparable, again indicating inhibition of N_2O reduction by nitrate.

Table 5.7. Estimates of K_2/K_1 from incubation of soil with no C_2H_2 or N_2O.

Depth (cm)	K_2/K_1 for soil from	
	Control Plot	Slurried Plot
0-10	25.9	1.25
10-20	39.3	2.41
20-30	61.6	38.3

5.5 Conclusions

In an atmosphere of C_2H_2, N_2O reduction was completely inhibited for at least 10 days and recovery of NO_3^- as N_2O was complete. Nitrate reduction began immediately but rates of reduction took several hours to reach a maximum, the time and maximum rate depending on the initial NO_3^- concentration.

While the data for both NO_3^- and N_2O reduction did not completely fit first order or Michaelis-Menten kinetics, there was evidence of substrate concentration dependence, probably because rates were limited by diffusion. Rates of NO_3^- reduction decreased more sharply with depth than rates of N_2O reduction.

From the incubation of soil in the absence of C_2H_2, for soil below 20cm, denitrification (indicated by NO_3^- disappearance) took place even though virtually no N_2O was detected, since rates of N_2O reduction were higher than for NO_3^- reduction. However, high N_2O concentrations were found in the incubation of surface soil, especially from the slurried plot, probably because of the inhibition by NO_3^- of N_2O reduction.

6. THE EFFECTS OF C_2H_2 ON SOIL PROCESSES.

It was intended to introduce C_2H_2 into the soil in enclosed microplots in the field once a week so that measurement of the N_2O flux from the soil at that time would give an estimate of total denitrification. Therefore, prior to setting up the field experiment, the effects of C_2H_2 on the soil were investigated.

Since C_2H_2 affects processes such as nitrification as well as denitrification (see Section 1.2.4) experiments were designed to determine the effects of C_2H_2 on mineralisation, nitrification and N_2O reduction, and whether any effects which occurred were reversible.

6.1 Soil

The soil used in all the experiments was from the 0-20cm depth from the field site described in Section 2.1, and had been collected in July 1980, passed through a 2mm sieve and stored field moist (0.13ml g^{-1} dry soil) at 4°C for about 7 months.

6.2 Mineralisation and Nitrification of N in the Presence and Absence of C_2H_2.

Experiment 1.

The incubation procedure was similar to that described by Bremner (1965b), to give an index of N availability. To each of 6 Erlenmeyer flasks (volume 500ml) containing 10g soil and 30g sand, 6ml of water were added, and 3 other flasks containing only water and sand were also prepared to act as controls. This ratio of sand, soil and water ensured good aeration and optimum moisture content for mineralisation and nitrification regardless of the moisture release characteristics of the soil. The acid washed sand was first boiled in alkali to remove any NH_4^+, and then rinsed well until all alkali was removed, the final rinsing being in double distilled water. The flasks were fitted with subaseal rubber stoppers, and 25ml of C_2H_2 added to three of the flasks containing soil, after 25ml

of air had been removed, giving an C_2H_2 concentration of about 0.04ml ml^{-1}. Since C_2H_2 contains about 2% acetone by volume, and at high concentrations acetone is known to inhibit nitrification (Hooper and Terry, 1973) acetone was removed by passing the gas from the cylinder through water. The incubation was carried out for 2 weeks at 30°C and the flasks were periodically sampled for CO_2, O_2 and N_2O analysis (Appendix 3.B). The soils were then analysed for NH_4^+ and NO_3^- (see Appendix 6 - NH_4^+ analysis in all experiments in Chapter 5 was by Method 2).

Where no C_2H_2 was added (Table 6.1) the increase in total inorganic N was almost all in the form of NO_3^-, indicating rapid nitrification, but in the presence of C_2H_2, NH_4^+ increased to very high concentrations while NO_3^- actually decreased, indicating that, at 0.04 ml ml^{-1}, C_2H_2 inhibited nitrification virtually completely. The presence of C_2H_2 also reduced the increase in total inorganic N during the incubation by more than half, thus it apparently had a significant effect on mineralisation also.

Table 6.1. Inorganic N in aerobic incubation in presence and absence of C_2H_2.

Form of inorganic N in soil	Inorganic N in Soil (µg-N g^{-1})			Change in Inorganic N in soil (µg-N g^{-1})	
	Before Incubation	After Incubation			
		$+C_2H_2$	$-C_2H_2$	$+C_2H_2$	$-C_2H_2$
NH_4^+	1.0	15.6	0.9	+14.6	- 0.17
NO_3^-	25.6	19.6	47.3	- 6.0	+21.7
Total	26.6	35.2	48.2	+ 8.6	+21.6

Experiment 2.

Flasks were prepared as in Experiment 1, with 3 control flasks, 5 flasks without C_2H_2 and 8 flasks with 0.05ml ml^{-1} C_2H_2, and following a preincubation at 14°C for 4 weeks 2 flasks with C_2H_2 and 2 without were

analysed for NH_4^+ and NO_3^- (Appendix 6), while all remaining flasks were flushed with air and the stoppers were replaced. To three of the flasks preincubated with C_2H_2, 25ml of C_2H_2 was added and all flasks were incubated at 30°C for two weeks. All the soil was then analysed for NH_4^+ and NO_3^-. During both incubations flasks were sampled periodically for CO_2, O_2 and N_2O analysis.

In the absence of C_2H_2 (Table 6.2) the increase in total inorganic N was largely in the form of NO_3^- following both the period of time at 14°C and at 30°C. For soil exposed to C_2H_2 throughout, NO_3^- decreased and NH_4^+ increased during the period at 14°C and there was no overall increase in total inorganic N but after 2 weeks at 30°C both NH_4^+ and NO_3^- had decreased almost to zero. For soil incubated at 30°C without C_2H_2 the release of N was slightly greater and nitrification much greater for soil pretreated with C_2H_2 than for soil pretreated without C_2H_2. The effect of C_2H_2 on nitrification was therefore reversible.

Table 6.2. Inorganic nitrogen in incubation following a preincubation with and without C_2H_2.

Form of Inorganic N	Inorganic N in soil (μg N g^{-1})					
	Initially	Following Preincubation at 14°C		Following Incubation at 30°C		
				Pretreated $+C_2H_2$		Pretreated $-C_2H_2$
		$+C_2H_2$	$-C_2H_2$	$+C_2H_2$	$-C_2H_2$	$-C_2H_2$
NH_4^+	0.7	8.0	0.0	1.0	0.2	0.1
NO_3^-	25.7	18.5	41.9	3.5	49.2	59.0
Total	26.4	26.5	41.9	4.5	49.4	59.1

Gas analysis showed O_2 concentrations above 0.18ml ml^{-1} and N_2O below 1 x 10^{-6} ml ml^{-1} during both experiments, i.e. all flasks were aerobic throughout the experiment. In both Experiment 1, and in the preliminary incubation at 14°C in Experiment 2, CO_2 concentrations increased more quickly in flasks treated with C_2H_2 (see Fig. 6.1). In the incubation at 30°C in Experiment 2 the difference became even more marked, but the preincubation with or without C_2H_2 made little difference to CO_2 production in flasks without added C_2H_2 (Fig. 6.2). The extra CO_2 formed indicates that C_2H_2 caused some sort of unusual microbial activity, which possibly led to a requirement for inorganic N, thereby decreasing NH_4^+ and NO_3^- during the incubation.

6.3. Effect on Mineralisation and Nitrification of Contact with C_2H_2 for 24h in each week.

The incubation method was similar to that described in Section 6.2, except that the temperature was 15°C, which was more typical of field temperatures.

After sealing with stoppers, sufficient C_2H_2 was added to 3 flasks to give a concentration of 0.005ml ml^{-1}, and to another 3 flasks to give a concentration of 0.05ml ml^{-1}, the other 3 acting as controls. After 24h C_2H_2 was removed by evacuating and filling with air 3 times, and then the flasks were incubated for a further 6 days. This procedure was repeated 4 times and at the end of the final 6 day incubation the flasks were analysed for CO_2, O_2 and C_2H_2 (Appendix 3B), and then for NH_4^+ and NO_3^- (Appendix 6).

In the control flasks there was a slight increase in NO_3^- concentrations and a slight reduction in NH_4^+, but the overall increase in total inorganic N was not large because of the low temperature of incubation (Table 6.3). At 0.005ml ml^{-1} C_2H_2 concentration both NH_4^+ and NO_3^- concentrations decreased slightly, while at 0.05ml ml^{-1} virtually all inorganic N had disappeared by the end of the experiment. Thus even though the C_2H_2 was in contact with the soil for only 1 day per week, at 0.005ml ml^{-1} C_2H_2 mineralisation and nitrification were slightly inhibited and at 0.05ml ml^{-1} almost

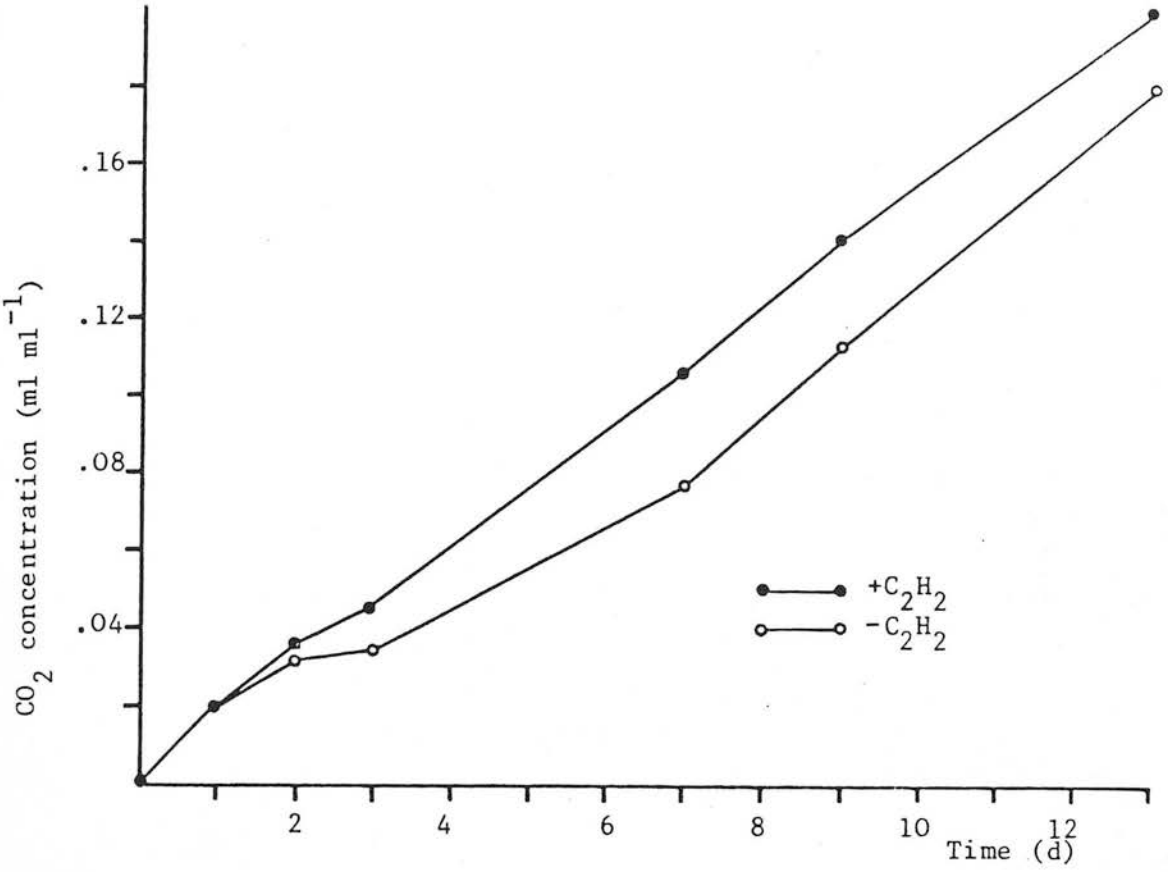


Fig. 6.1. CO_2 concentrations in flasks in incubation with and without C_2H_2

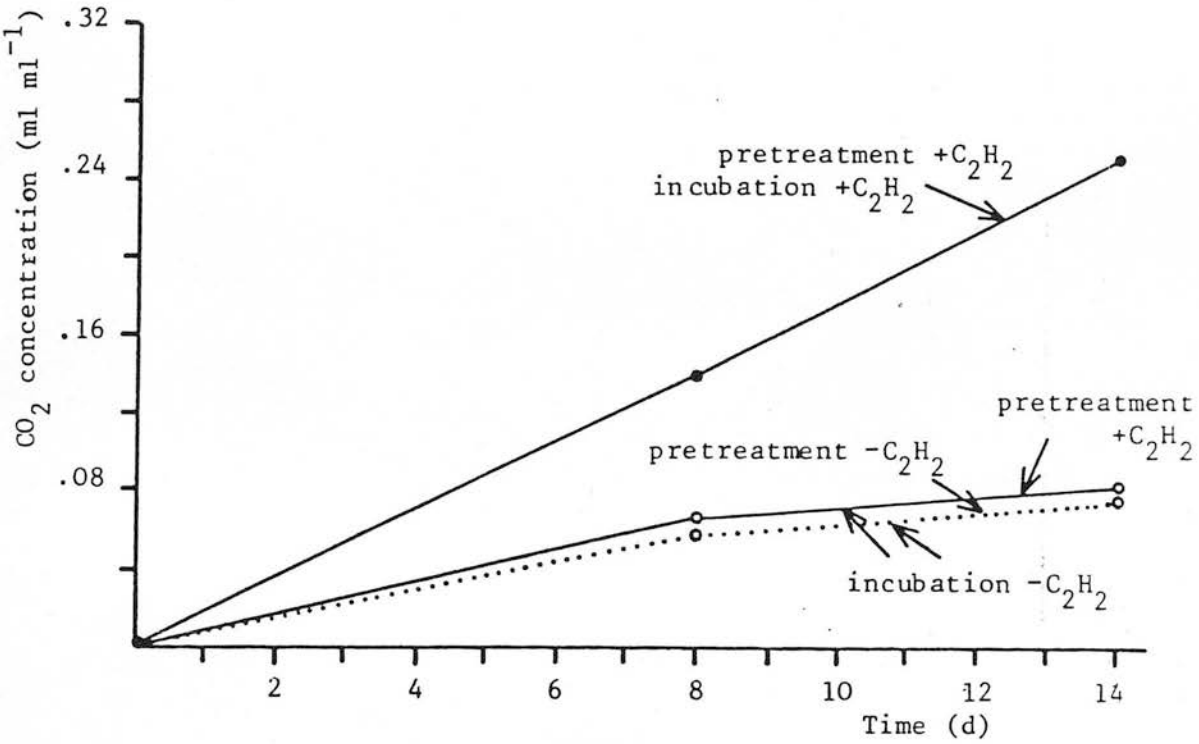


Fig. 6.2. CO_2 concentrations in flasks following preincubation with and without C_2H_2

totally inhibited so that N was taken up by micro-organisms faster than it could be supplied by mineralisation.

At the end of the experiment, following a 6 day period without C_2H_2, O_2 concentrations in all flasks were $>$ 0.19ml ml^{-1} showing that the flasks remained aerobic. No C_2H_2 was detected in any of the flasks (limit of detection 0.001ml ml^{-1}). Concentrations of CO_2 were over 7 times higher in flasks treated with 0.05ml ml^{-1} C_2H_2 than in the control flasks. Thus there was evidence of stimulation of microbial activity by C_2H_2 (which perhaps gave rise to a high demand for inorganic N) the effect continuing even when C_2H_2 was removed.

Table 6.3. Inorganic N following incubation with C_2H_2 for 1 day per week.

Form of Inorganic nitrogen	Inorganic nitrogen in the soil (μg N g^{-1})			
	Initially	Following incubation with		
		no C_2H_2	0.005ml ml^{-1} C_2H_2	0.05ml ml^{-1} C_2H_2
NH_4^+	10.1	6.5	8.3	2.9
NO_3^-	64.2	72.4	62.2	1.9
Total	74.3	78.9	70.5	4.8

6.4. Effect of C_2H_2 at 2 Concentrations on N_2O Reduction.

Nine 500ml Erlenmeyer flasks, each containing 10g soil and 20ml of water were fitted with a Quickfit adjustable Drechsel head, in which the gas inlet tube had been replaced by a silicone septum, and the outlet tube connected to a 3-way stopcock (Fig. 6.3). The flasks were evacuated and filled with N_2, through the 3-way stopcock, 3 times. To 3 flasks C_2H_2 was then added to give a concentration of 0.005ml ml^{-1} and to 3 to give a concentration of 0.05ml ml^{-1}. The

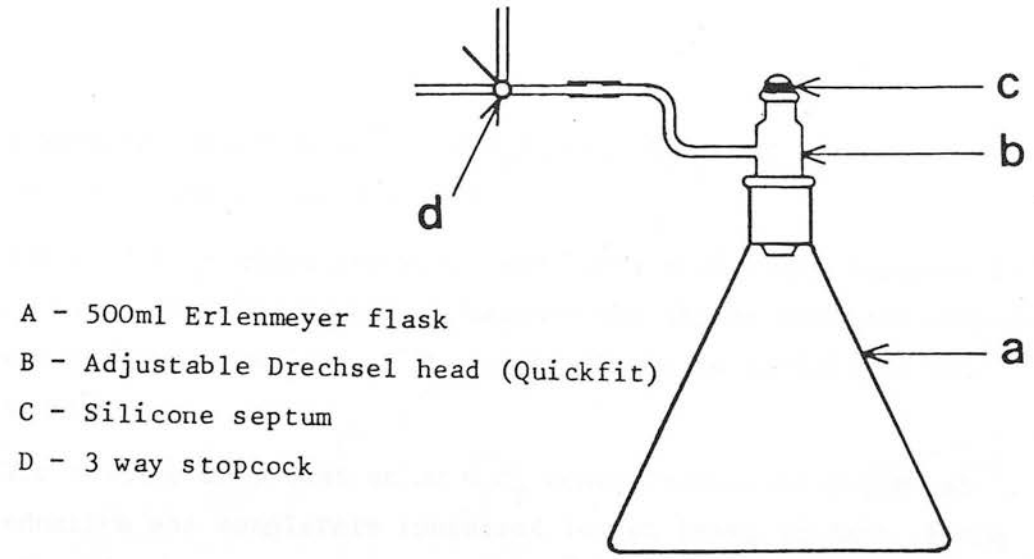


Fig. 6.3. Flasks used in incubation experiment

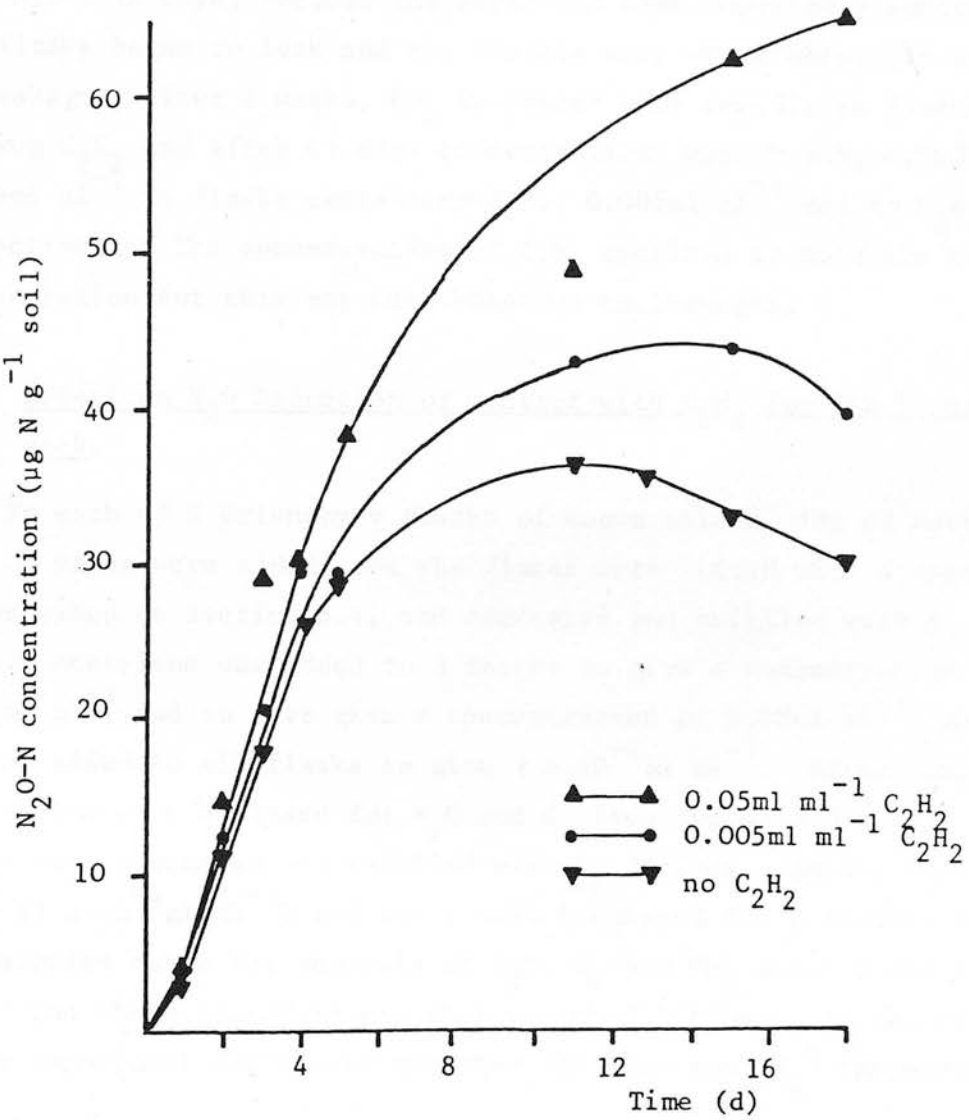


Fig. 6.4. N_2O concentrations in incubation with 3 concentrations of C_2H_2

flasks were incubated at 30°C and N_2O, CO_2, C_2H_2 and O_2 concentrations were measured periodically.

Rates of NO_3^- reduction were much lower than those reported in Section 5.4. This may have been because the flasks were not completely anaerobic; concentrations of O_2 in the flasks up to 0.017ml ml^{-1} were measured.

The results show that at an C_2H_2 concentration of 0.05ml ml^{-1}, N_2O reduction was completely inhibited for at least 18 days, since the N_2O maximum corresponded to the NO_3^- initially present in the soil (62.6µg N g^{-1}), while at 0.005ml ml^{-1}, C_2H_2 was only partially effective (Fig. 6.4).

After 18 days, because the septa had been repeatedly punctured, the flasks began to leak and the results were unreliable. In spite of leakages, after 2 weeks, CO_2 increased more rapidly in flasks containing C_2H_2 and after 41 days concentrations were 0.115, 0.063 and 0.056ml ml^{-1} in flasks containing 0.05, 0.005ml ml^{-1} and no C_2H_2 respectively. The concentration of C_2H_2 declined to half the original concentration but this may have been due to leakages.

6.5. Effect on N_2O Reduction of Contact with C_2H_2 for 24h in each week.

To each of 9 Erlenmeyer flasks of known volume, 10g of soil and 20ml of water were added, and the flasks were fitted with stoppers as described in Section 6.4, and evacuated and refilled with N_2 3 times. Acetylene was added to 3 flasks to give a concentration of 0.005ml ml^{-1} and to 3 to give a concentration of 0.05ml ml^{-1}, and N_2O was added to all flasks to give 1×10^{-4}ml ml^{-1}. After 24h, gas samples were analysed for N_2O and O_2 (see Appendix 3B) and flasks were evacuated and refilled with N_2 3 times. Again, N_2O was added (1×10^{-4}ml ml^{-1}) and the flasks incubated for a further 6 days with samples taken for analysis of N_2O, O_2 and CO_2 after 2 and 6 days. The whole procedure was then repeated 5 times. At the end of the experiment soils were analysed for NO_3^- and NH_4^+ (Appendix 6).

During the first 2 cycles, on days when C_2H_2 was present, NO_3^- already present in the soil was reduced so that N_2O increased during the 24h. It is clear from Table 6.4 that 0.005ml ml^{-1} C_2H_2 did not totally inhibit N_2O reduction since N_2O was lower than in flasks containing 0.05ml ml^{-1} C_2H_2. In the following weeks, N_2O reduction was less in flasks containing C_2H_2 but inhibition was not complete even at the higher level. However, there was no evidence of any further lessening of the inhibiting effect of C_2H_2 after 6 weeks.

During the first 6 day incubation, between successive C_2H_2 additions, N_2O concentrations increased during the week, but in the following weeks N_2O decreased, with flasks which had been treated with C_2H_2 having higher N_2O concentrations than the controls (Table 6.5). The results show, therefore, that the effect of C_2H_2 was not immediately reversible. For flasks which had been treated with the lower C_2H_2 concentration (but not for flasks with the higher concentration), during the last 4 days of the incubation N_2O reduction was similar to that in the control flasks, i.e. after 2 days the micro-organisms recovered the ability to reduce N_2O.

No O_2 was detected in any of the flasks during the incubation. In contrast to previous experiments there was no difference in CO_2 concentrations after the 6 day periods without C_2H_2.

No NO_3^- remained in any of the flasks at the end of the experiment but NH_4^+ concentrations had increased from 4µg N g^{-1} to 21µg N g^{-1} in all treatments, therefore the presence of C_2H_2 for 1 day per week did not affect anaerobic mineralisation.

Table 6.4. N_2O concentrations after 24h incubation with N_2O and C_2H_2 at 3 concentrations, in successive weeks.

Week	N_2O remaining in flasks (% of initial concentration)		
	No C_2H_2	0.005ml ml^{-1} C_2H_2	0.05ml ml^{-1} C_2H_2
1	207	249	284
2	111	128	134
3	69	93	90
4	82	99	101
5	76	78	87
6	71	89	92

Table 6.5. N_2O concentrations after 6 day incubation with N_2O with no added C_2H_2

Week	Day	N_2O remaining in flasks (% of initial concentration)		
		No C_2H_2	0.005ml ml^{-1} C_2H_2	0.05ml ml^{-1} C_2H_2
1	2	309	357	351
	6	268	545	544
2	2	64	76	86
	6	26	44	73
3	2	64	90	98
	6	17	47	79
4	2	66	87	80
	6	30	69	80
5	2	32	64	85
	6	9	43	76

6.6. Conclusions

At concentrations of 0.04ml ml^{-1}, C_2H_2 completely inhibited nitrification, reduced aerobic mineralisation, and caused a build up of NH_4^+. The effect on the soil of contact with C_2H_2 at 0.04ml ml^{-1} for 4 weeks was reversible and in fact nitrification was then faster because NH_4^+ had built up. Unexpectedly, after prolonged contact with C_2H_2, concentrations of NO_3^- and NH_4^+ became very low.

When aerobic soil was in contact with C_2H_2 for 1 day a week for 5 weeks at a concentration of 0.005ml ml^{-1}, there was little difference in release of inorganic N compared with the control receiving no C_2H_2. At 0.05ml ml^{-1} C_2H_2 for 1 day per week, concentrations of both NH_4^+ and NO_3^- decreased.

In an anaerobic experiment 0.05ml ml^{-1} C_2H_2 was sufficient for complete inhibition of N_2O reduction for 2 weeks, but 0.005ml ml^{-1} was only partially effective. At the lower concentration applied for 1 day per week, the effect of C_2H_2 was reversible after 2 days but at the higher concentration N_2O reduction was at least partially inhibited for 6 days after C_2H_2 had been removed.

In all the experiments reported here, except Section 6.5, CO_2 production was higher in soil treated with C_2H_2, indicating that C_2H_2 stimulated microbial activity. In one experiment there was evidence of C_2H_2 concentrations decreasing (Section 6.4) but in other experiments the sensitivity of the analysis did not allow small decreases in C_2H_2 concentrations to be demonstrated. Recently reports have appeared of C_2H_2 acting as a substrate for certain (rare) microorganisms (see Section 1.2.4.2). It is possible therefore that the extra CO_2 was formed by the oxidation of C_2H_2. This may also account for the large decrease in inorganic N during some incubations, since if a population of C_2H_2 oxidisers grew rapidly, they would generate a demand for inorganic N.

The implications for the use of C_2H_2 in field experiments are as follows:

- nitrification may be affected by the use of C_2H_2.
- concentrations of C_2H_2 should be >0.005ml ml^{-1} for effective inhibition of N_2O reduction.
- C_2H_2 may continue to have an effect on N_2O reduction for at least 6 days after its use.
- CO_2 concentrations may increase as a result of the use of C_2H_2.

7. INVESTIGATIONS OF THE ABILITY OF C_2H_2 TO SUPPORT MICROBIAL GROWTH.

Incubation experiments had shown that C_2H_2 increased CO_2 production in aerobic conditions while large quantities of inorganic N, and possibly some C_2H_2 disappeared (Chapter 6). Recent reports (see Section 1.2.4.1) suggested that there may be a species of bacterium in the soil capable of using C_2H_2 as a C source. Soil incubation, agar plate culture and liquid culture experiments were designed to test whether C_2H_2 was able to support the growth of microorganisms from soil, and what kinds of microorganisms were responsible.

7.1 Soil Incubation

Soil from the field plot (as described in Section 6.1) was incubated with and without C_2H_2 in the presence of solutions of inhibitors of biological activity or water alone. If the effect of C_2H_2 were chemical, inhibitors would make little difference, while if it were biological, they would be expected to reduce it.

To each of 16 500ml Erlenmeyer flasks of precisely known volume, 20g soil and 60g of acid washed sand were added and mixed, followed by 11ml of distilled water. To each group of 4 flasks, 1ml of one of the following solutions or water as a control was added:-

1) 10^{-2}M potassium fluoride (prevents the formation of pyruvate from glucose and therefore inhibits respiration of all organisms).
2) 1g l^{-1} chloramphenicol (inhibitor of protein synthesis in most bacteria).
3) 1g l^{-1} cycloheximide (inhibits protein synthesis of fungi, algae and protozoa).

The moisture content was around field capacity - the optimum for aerobic microbial activity. All flasks were sealed with subaseals, and 30ml of air was replaced by 30ml C_2H_2 by syringe in 2 flasks from each treatment. All flasks were incubated at 30°C for 6 weeks, and

gas samples taken at intervals for CO_2, C_2H_2 and O_2 analysis (see Appendix 3B). Four weeks after the incubation, flasks were reflushed and sealed and a further 20ml of C_2H_2 were added to flasks previously treated with C_2H_2; gas concentrations were monitored over the next few days.

In flasks to which C_2H_2 was added, for all treatments, CO_2 production was greater and C_2H_2 concentrations decreased to zero (Fig. 7.1). Flasks containing fluoride, with and without C_2H_2, showed similar concentrations of C_2H_2 and CO_2 to the control. In the presence of C_2H_2, enhanced CO_2 production began after about 13 days in the fluoride treatment and the control, but at 20 and 10 days with chloramphenicol and cycloheximide respectively. The rate of C_2H_2 consumption was greater with cycloheximide, all C_2H_2 disappearing by 17 days, suggesting that C_2H_2 was consumed by bacteria rather than fungi.

The lag period prior to C_2H_2 consumption suggests either that the few organisms responsible, which were present initially, grew rapidly in numbers after a few days or that adaptation of existing organisms took a similar time. The rate of CO_2 production in C_2H_2 treated flasks always corresponded to C_2H_2 consumption, but only half as much extra CO_2-C was produced as C_2H_2-C was consumed, suggesting that C_2H_2 was being incorporated into microbial cells as well as being used for respiration. The decrease in O_2 (about 0.07ml ml^{-1}) in flasks with C_2H_2 compared to those without, was almost the same as the increase in CO_2 (about 0.06ml ml^{-1}) at the time when C_2H_2 had disappeared. The lowest O_2 concentration measured was 0.07ml ml^{-1}. This gives further proof that the process is aerobic.

When the second C_2H_2 addition was made, C_2H_2 concentrations began to fall after 1 day in all flasks, and after 10 days was zero, except in the flasks treated with chloramphenicol. Thus the population of microorganisms which used C_2H_2 retained the ability even after 4 weeks without C_2H_2.

The experiment clearly demonstrated that a large population of microorganisms able to utilise C_2H_2 as a C source built up in

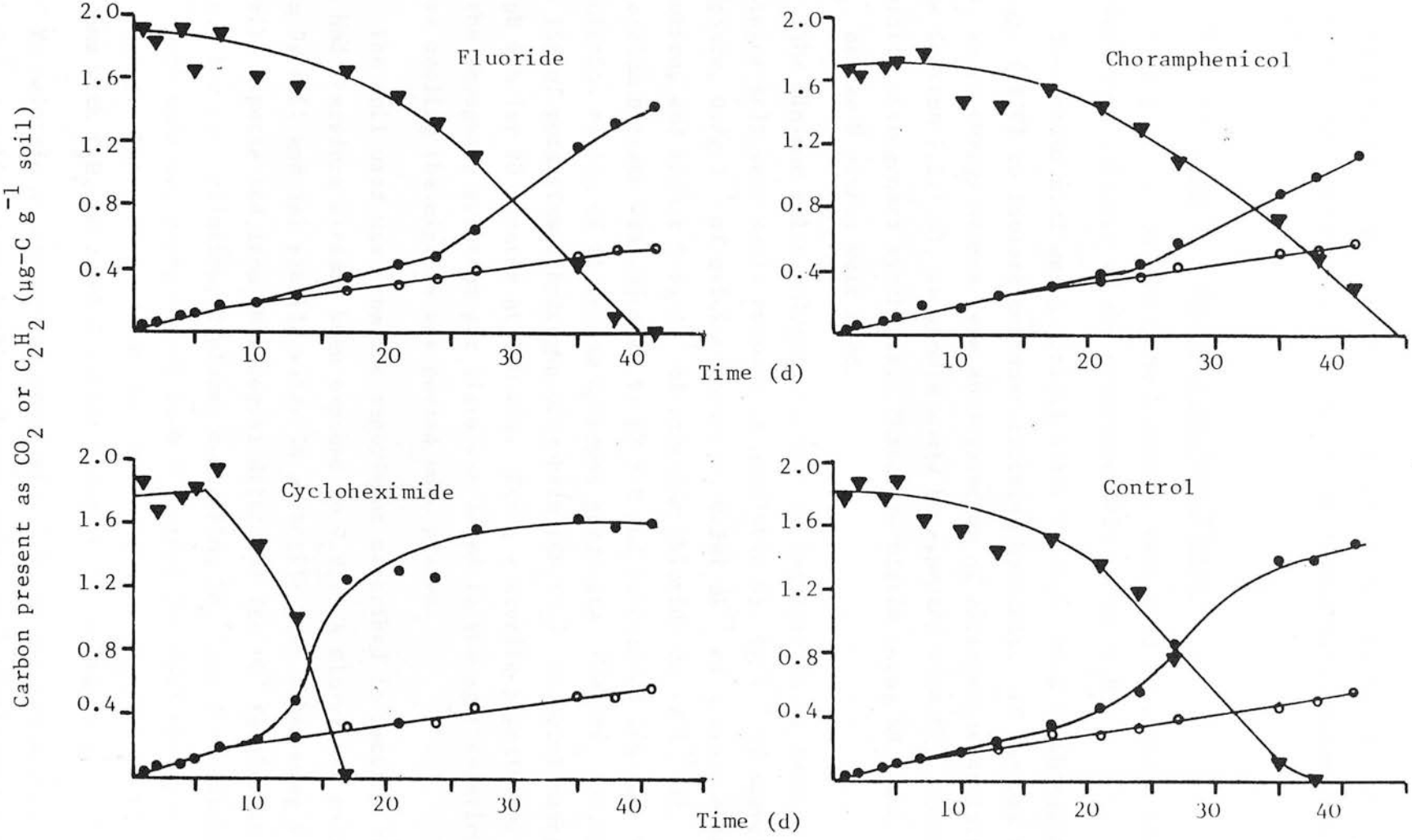


Fig. 7.1. CO_2 production and C_2H_2 consumption in an incubation experiment

aerobic soil and that probably the organisms were bacteria. The long lag period may explain why the effect has seldom been noticed previously.

7.2 Incubation of Soil Extracts on Agar Plates

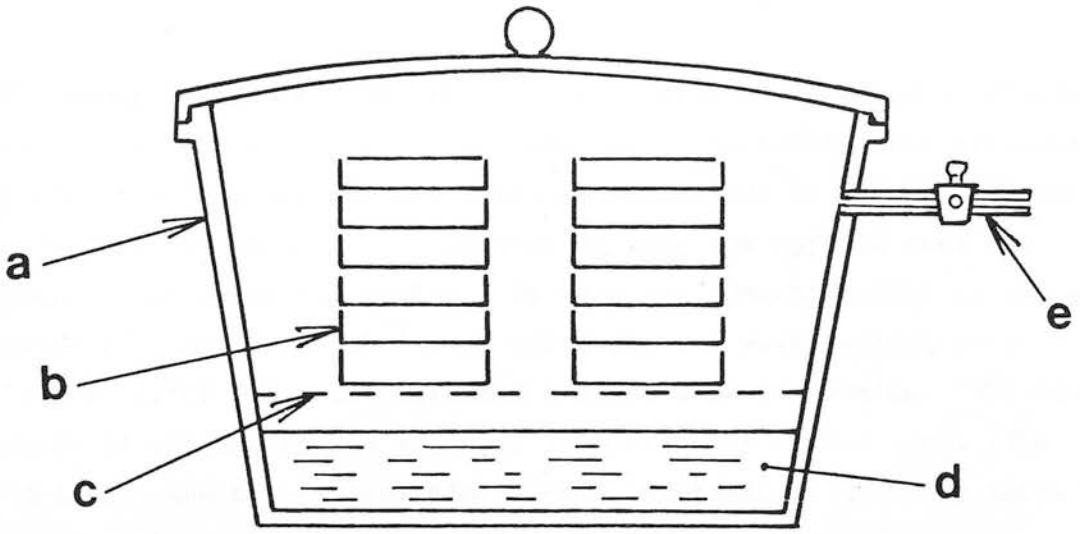
Agar plates, containing no C source, were used to attempt to isolate pure cultures of the organisms able to use C_2H_2.

The medium used was a minimal salts solution used by Whittenbury et al. (1970) to isolate methane-utilising bacteria. If the use of C_2H_2 as an energy source were an adaptation of nitrogenase activity (see Section 1.2.4.4), no growth would be expected with NH_4^+ which inhibits nitrogenase synthesis. Therefore plates using NH_4^+ and NO_3^- as the N source were used.

The minimum salts solution containing 15g Agar No. 1 (which contains only very small amounts of available C), 1g l^{-1} of magnesium sulphate, 0.2g l^{-1} of calcium chloride, 0.5ml ml^{-1} of a trace element solution, and either 0.5g l^{-1} of ammonium chloride or 1g l^{-1} of potassium nitrate was adjusted to pH 6.8 and autoclaved together with a solution of 15g of di-sodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) and 15g of potassium dihydrogen phosphate (KH_2PO_4) in 300ml water at pH 6.8 for 20 minutes at 100 kPa. Using a sterile pipette 2ml of the phosphate solution per litre was added to the agar solution. After cooling the mixture was poured onto plates.

The soil used was from the experiment described in Section 7.1 and had therefore already been exposed to C_2H_2. A slurry was made from 3g soil and 3ml sterile water in a sterile test tube using a sterile pipette and, from this serial dilutions (to 10^4 fold) were made. For each dilution, 2 plates containing NH_4^+ and 2 containing NO_3^- were made up, using a wire loop to spread the soil slurry over the plates. One replicate was incubated without C_2H_2 as a control, and one with C_2H_2 in a metal vessel illustrated in Figure 7.2.

To maintain an atmosphere containing C_2H_2 in the container, C_2H_2 from a cylinder was bubbled through water in a Winchester bottle



a - metal container with tightly fitting lid

b - agar plates

c - perforated metal plate

d - acetylene saturated water

e - sampling outlet

Fig. 7.2. Container used for incubating agar plates with C_2H_2

for about 10 minutes, and the C_2H_2 saturated water (solubility > 1ml ml^{-1}) was then poured into the empty metal container, the perforated plate replaced, and the agar plates quickly put in place, followed by the greased lid. Insulating tape was applied over the joint as an extra precaution. In this way concentrations in the gas phase of about 0.1ml ml^{-1} were achieved, the water acting as a 'reservoir'of C_2H_2, sufficient for a 2-3 week incubation. The atmosphere in the vessel was analysed regularly for O_2 and C_2H_2. The container and the control plates were incubated at 30°C for three weeks.

After 3 weeks each colony type was streaked onto new plates of the same N source, as far as possible, without fungal contamination, and the plates were again incubated with C_2H_2 for 2 weeks. The same minimal salts solution was used but the agar was first washed in distilled water to remove any soluble C compounds present.

The colonies were streaked out twice more in an attempt to isolate pure cultures, once as described above and once with each colony plated onto a medium with and without cycloheximide (a fungal inhibitor) added to the salts solution.

The greatly increased growth in plates exposed to C_2H_2 (Plate 7.1) could possibly have been due to carbon containing impurities; acetone (up to 2%) and traces of CH_4 and CO. However, most of the acetone had been removed by passing C_2H_2 through water and traces of CH_4 and CO would be insufficient to cause so much growth.

The amount of growth was dependent on the dilution used, showing that the organisms originated from the soil and not from contamination e.g. from the salts solution or the vessel. Growth on NO_3^- was slightly greater than on NH_4^+. On the control plates there were few colonies especially at high dilutions, although slightly more occurred with NH_4^+ than with NO_3^-. The growth was probably due to traces of available C substrates in the agar and to soil organic matter.

On each plate there was more than one colony, differing often

Plate 7.1. Growth on agar plates incubated with and without C_2H_2

in shape and colour, and on most of the plates there was a good deal of fungal growth, mostly in association with bacterial colonies. Observation under the microscope showed that there was a wide variety of bacteria on the plates and that most colonies were not pure cultures. There were colonies of the same appearance on plates with NO_3^- and NH_4^+.

After the second incubation, there was still more than one colony type per plate and a lot of fungal growth. The fungi always grew with the bacteria but bacterial colonies often grew alone, indicating that the fungi were parasitic on the bacteria. Again there was slightly more growth on NO_3^- than NH_4^+.

During the successive plating out of the cultures, the colonies sometimes changed colour and shape, possibly due to ageing or to different bacteria in a mixed culture becoming dominant. Examination under a microscope, after the final incubation, showed that some cultures were still not composed of a single organism. Generally cycloheximide reduced fungal growth but inhibition was not complete.

Since the fungi seemed to be parasitic on the bacteria, it was almost certainly the bacteria which were able to use C_2H_2 (Bacteria are known to be more adaptive to unusual environments than other microorganisms).

Although pure cultures were not isolated the experiment demonstrated the ability of some bacteria to grow on C_2H_2. It is possible that in the associations, some bacteria were living on the products of the C_2H_2 utilizers.

Whereas previous evidence has indicated that only very few bacteria can use C_2H_2 as a C source (see Section 1.2.4.2) at the end of the series of incubations described here, there were at least 7 different cultures or associations on the plates, originating from one soil.

7.3 Liquid Culture

The experiments were intended to prove, beyond any reasonable doubt, that the bacteria from the agar plate experiment consumed C_2H_2.

A description of the colonies chosen, through each of the agar plate incubations is given in Table 7.1. The 300ml flasks used were autoclaved after adding 50ml of the minimal salts medium described in Section 7.2 (but agar was omitted). The cultures chosen were inoculated into flasks in duplicate (all of them without fungus) using a sterile wire loop, and sealed with sterile subaseals. Flasks contained the same N source on which the culture had been grown previously. In one of the duplicate flasks 25ml of air were replaced by 25ml of C_2H_2. All flasks were shaken at 30°C and were periodically analysed for CO_2, O_2 and C_2H_2 (Appendix 2) and for C_2H_4 by gas chromatography because if C_2H_2 were metabolised by a nitrogenase type reaction then C_2H_4, the reduction product, would be detectable. Hydrocarbons were separated at 110°C on a column of alumina (60-80 mesh, partially deactivated with sodium iodide), using N_2 as a carrier gas (75ml ml^{-1}), and gas concentrations were measured with a flame ionisation detector.

In a second experiment, the same procedure was followed except that 0.1g l^{-1} of yeast extract was added to the salts solution to provide any vitamins the organisms might require. The addition of some C compounds in the yeast extract was enough to give only limited growth. One flask, containing culture 1 but no C_2H_2, and a flask containing the medium and C_2H_2 but no culture acted as controls. One flask was inoculated with culture 1 from the 1st liquid culture experiment to make sure it would continue to grow.

In the first experiment growth was rapid with culture 1 after only 1 day, 75ml of C_2H_2 being consumed in 2½ weeks, but was slight in other flasks, CO_2 concentrations being no greater than in the controls (probably due to traces of C compounds in the mineral salts solution). The bacteria may have required vitamins normally present in agar or a surface on which to grow. Sometimes, in liquid

Table 7.1. Description of Colonies Chosen for Liquid Culture Incubation following 4 Solid Plate Incubation

No. and nitrogen source	Appearance of Colony Following each Incubation				Growth in Liquid Culture	
	1st	2nd	3rd	4th	Experiment 1	Experiment 2
1 (NO_3^-)	Small white	Small white	Creamy irregular shaped with yellowish centre	Pinkish irregular shaped	Rapid growth	Rapid growth
2 (NO_3^-)	Yellowish mucoid	Yellowish mucoid	Yellowish mucoid	Yellowish mucoid	No growth	Rapid growth
3 (NO_3^-)	Yellowish	Translucent	Yellowish small	Translucent	No growth	n.d.
4 (NH_4^+)	Tiny white round	Tiny white round	Tiny white round	Tiny white round	No growth	Little growth
5 (NH_4^+)	Buff coloured spreading	Buff coloured white centre	Buff coloured white centre	Buff coloured white centre	No growth	Good growth
6 (NH_4^+)	Small white round	Translucent spreading	Translucent spreading	Translucent spreading with white centre	No growth	Little growth
7 (NH_4^+)	Spreading greyish mucoid	Small pinkish irregular shape with white centre	Pinkish, irregular shape with white centre	Pinkish with white centre	n.d.	Good growth
8 (NH_4^+)	Greyish mucoid	Greyish mucoid	Greyish round mucoid	Greyish round mucoid	n.d.	Slow growth

n.d. - Not determined.

cultures, bacteria poison themselves, e.g. de Bont and Peck (1980) found poisoning with acetaldehyde, an intermediate in the metabolism of C_2H_2, to be a problem when they attempted to isolate C_2H_2 utilizers.

In the second experiment, there was virtually no growth (i.e. negligible CO_2 production) either in the flask containing medium and C_2H_2 but no culture (showing that the flasks were sterile) or in the flask containing medium and culture 1 but no C_2H_2. The final concentration in the latter flask (0.004ml ml^{-1}) indicated the amount of CO_2 which could be formed from the limited C substrates present. Of the cultures incubated with C_2H_2, however, only 4 and 6 failed to show much growth, CO_2 concentrations reaching only 0.005 and 0.012ml ml^{-1} respectively. In all other cultures, C_2H_2 was consumed, and decreases in C_2H_2 corresponded with increases in CO_2 (Fig. 7.3), proving beyond doubt that C_2H_2 and not some impurity was responsible for growth. Less CO_2 was produced (by volume) than C_2H_2 consumed (Table 7.2) and thus more than half of the C from C_2H_2 was converted to cell biomass, the rest being used for respiration. The consumption of O_2 was approximately the same as the production of CO_2 (by volume).

Table 7.2 Comparison of CO_2 production and C_2H_2 and O_2 consumption.

Culture	CO_2 produced[a] ($ml\ ml^{-1}$)	C_2H_2 consumed ($ml\ ml^{-1}$)	O_2 consumed ($ml\ ml^{-1}$)
1	6.6	8.3	7.3
2	7.5	7.8	8.5
5	5.0	5.6	5.4
7	4.9	5.3	4.5
8	2.6	4.8	2.6

[a] Calculated by subtracting CO_2 in the control (0.004ml ml^{-1}) from the final CO_2 concentration.

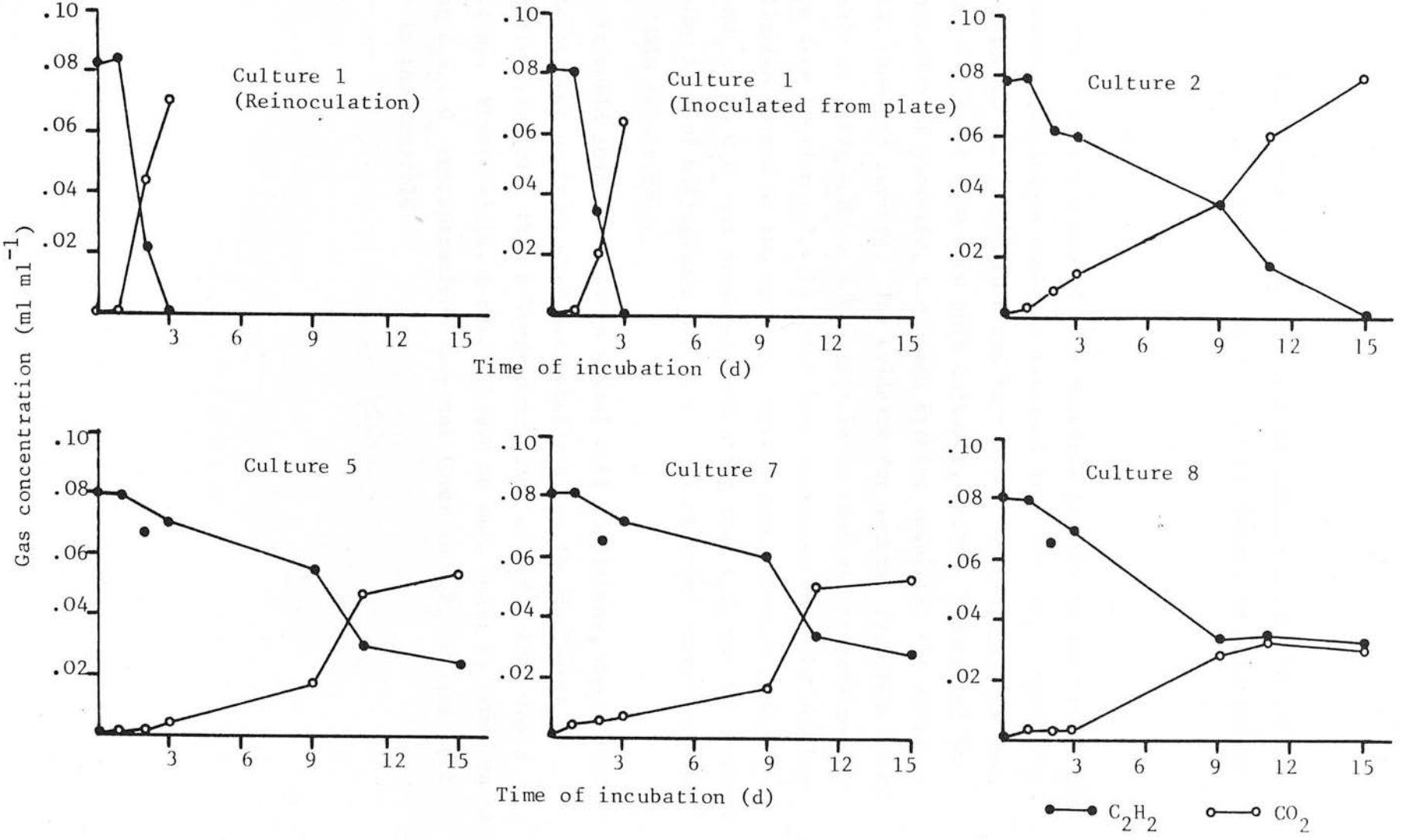


Fig. 7.3. Concentrations of CO_2 and C_2H_2 during liquid culture experiment

Concentrations of C_2H_4 remained at ambient levels in all flasks during the incubation, i.e. there was no evidence of nitrogenase activity.

7.4 Conclusions

The experiments showed that bacteria capable of utilizing C_2H_2 in aerobic conditions could be isolated from the soil. More than one organism was responsible and even after repeated plating out, colonies may not have been pure cultures, perhaps indicating an association of bacteria, i.e. each species requiring the presence of the others to survive. The evidence for several organisms being capable of using C_2H_2 as a C source is in contrast to previous reports (see Section 1.2.4.2) which have indicated that the ability is limited to one or two species. Growth occurred with both NH_4^+ and NO_3^-. No C_2H_4 was produced, indicating that C_2H_2 was not reduced by some kind of nitrogenase activity. All cultures except one had a vitamin requirement.

It would seem unlikely in normal soil conditions, where competition between bacteria does not usually allow the dominance of specific organisms, that a large population of C_2H_2 users would build up. Nevertheless, a check should be made that, in experiments using C_2H_2, O_2 concentrations are not lower in C_2H_2 treated soil than in the controls.

CHAPTER 8. FIELD EXPERIMENT TO MEASURE FLUXES OF N_2O

Methods of measurement of fluxes of N_2O and N_2 in the field have been discussed in Section 1.5.2. Total N flux resulting from denitrification can be measured either by using ^{15}N-enriched fertiliser and measuring the subsequent fluxes of N_2O and ^{15}N-labelled N_2, or by establishing C_2H_2 concentrations in the soil adequate to inhibit N_2O reduction so that all N lost by denitrification is in the form of N_2O. Since with the latter technique N loss from slurry can also be measured and the technique is sensitive to smaller losses, this was the method used in the experiment reported below.

8.1. Experimental Methods

An open canopy method was used (see Section 1.5.2. for a discussion of this and alternative methods) so that N_2O concentrations under the canopy were close to ambient while fluxes were being measured.

8.1.1. Enclosed Microplots

Small areas of soil were enclosed with lengths of rigid PVC cylindrical piping inserted vertically into the soil to form microplots isolated from the surrounding soil. This arrangement facilitated the establishment of adequate C_2H_2 concentrations in the soil profile. The cylinders had an internal diameter of 21.8cm, a wall thickness of 1cm and a length of 48cm. One end was chamfered to make a cutting edge. Holes for soil atmosphere samplers and tensiometer probes were drilled in the cylinder walls prior to insertion into the ground.

The cylinders were installed at one end of the field plot (described in Section 2.1) in an area not previously used either during the preliminary field experiment or the randomised block experiment. An access trench was dug, about 12m long by 1m wide by 0.5m deep. The cylinders were placed on end immediately adjacent to the edge of the trench (Fig. 8.1) and spaced out as shown in Fig. 8.2. Each cylinder in turn was covered with a thick piece of wood and slowly hammered (with a sledgehammer) vertically into the soil. The soil immediately surrounding the cylinder was dug away as it was driven downwards, to minimise impedance (Belford, 1979) and the process

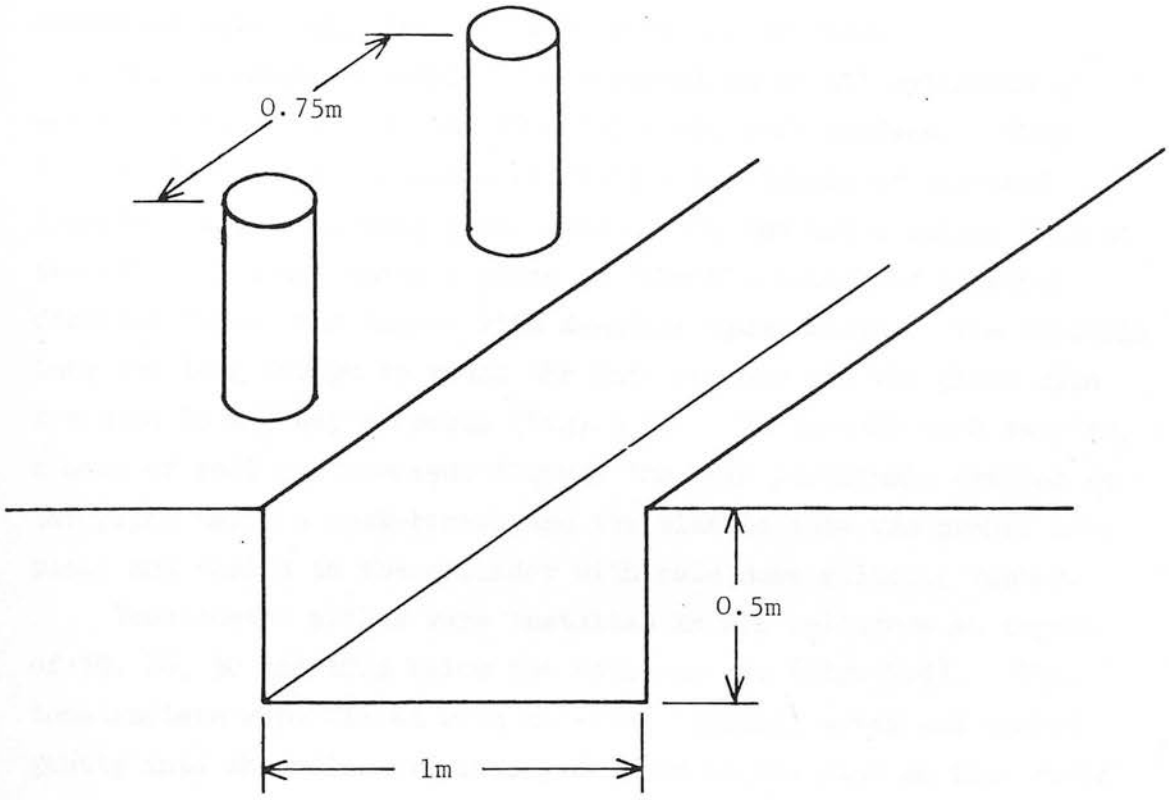


Fig. 8.1. Installation of the PVC cylinders

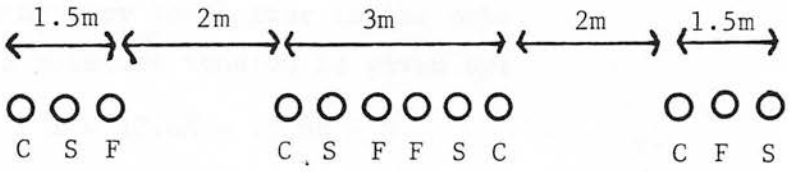


Fig. 8.2. Arrangement of microplots

Treatments: C = control, S = slurried, F = fertilised

continued until only 4cm was left above ground level.

Soil atmosphere samplers were installed in all cylinders at depths of 5, 10, 20, 30 and 40cm below the soil surface. They consisted of an 8cm length of rigid plastic tubing of internal diameter 1cm, containing glass wool at one end and a rubber bung at the other, through which a piece of flexible tubing of internal diameter 1.5mm was sealed with Araldite epoxy resin. The flexible tube was long enough to reach the soil surface and was glued with Araldite to a 3 way stopcock (Fig. 8.3). To install each sampler, a core of soil was removed, through the hole previously drilled in the pipe, using a cork-borer, and the plastic tube was pushed into place and sealed to the cylinder with cold cure silicone rubber.

Tensiometer probes were installed in all cylinders at depths of 10, 20, 30 and 40cm below the soil surface (Fig. 8.4). The tensiometers were filled with air-free (boiled) water and pushed gently into the holes previously drilled in the pipe so that there was good contact between the porous pot and the soil. Flexible tubing was pushed through the stopper and into the porous pot. By carefully injecting air free water through the side-arm of the rigid tube, the air in the flexible tubing was expelled from the other end, which dipped in a mercury reservoir and was fixed to a wooden post (Fig. 8.4). There was then an unbroken water column between the porous pot and the mercury reservoir, so that when the soil was under tension, the mercury level rose in the tube.

The soil moisture tension is given by:

$$T = 12.6M - 13.6R + H \qquad 8.1$$

where M is the height of mercury in the plastic tube (cm)
R is the height of mercury in the reservoir (cm)
H is the height of the porous pot (cm)
and height is measured from the soil surface with height above the ground positive

In November 1981, sets of tensiometers were installed in the undisturbed soil adjacent to each group of cylinders (i.e. 1-3,4-9, and 10-12), each set consisting of one probe at depths of 10, 20, 30 and 40cm

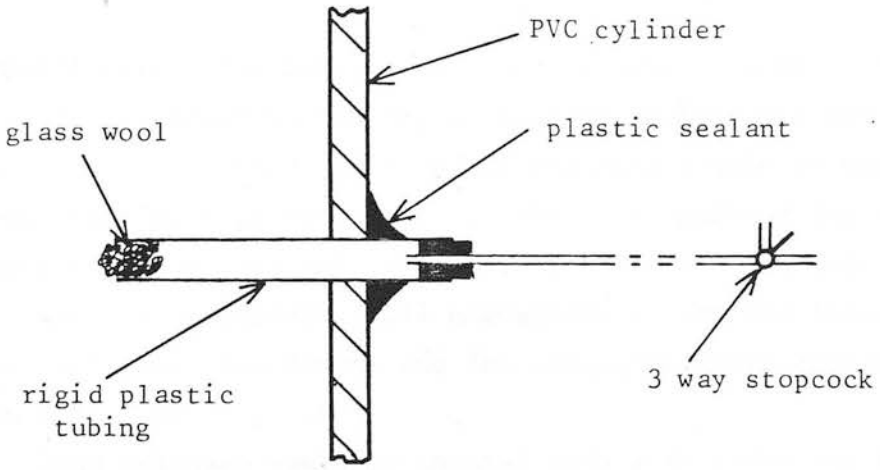
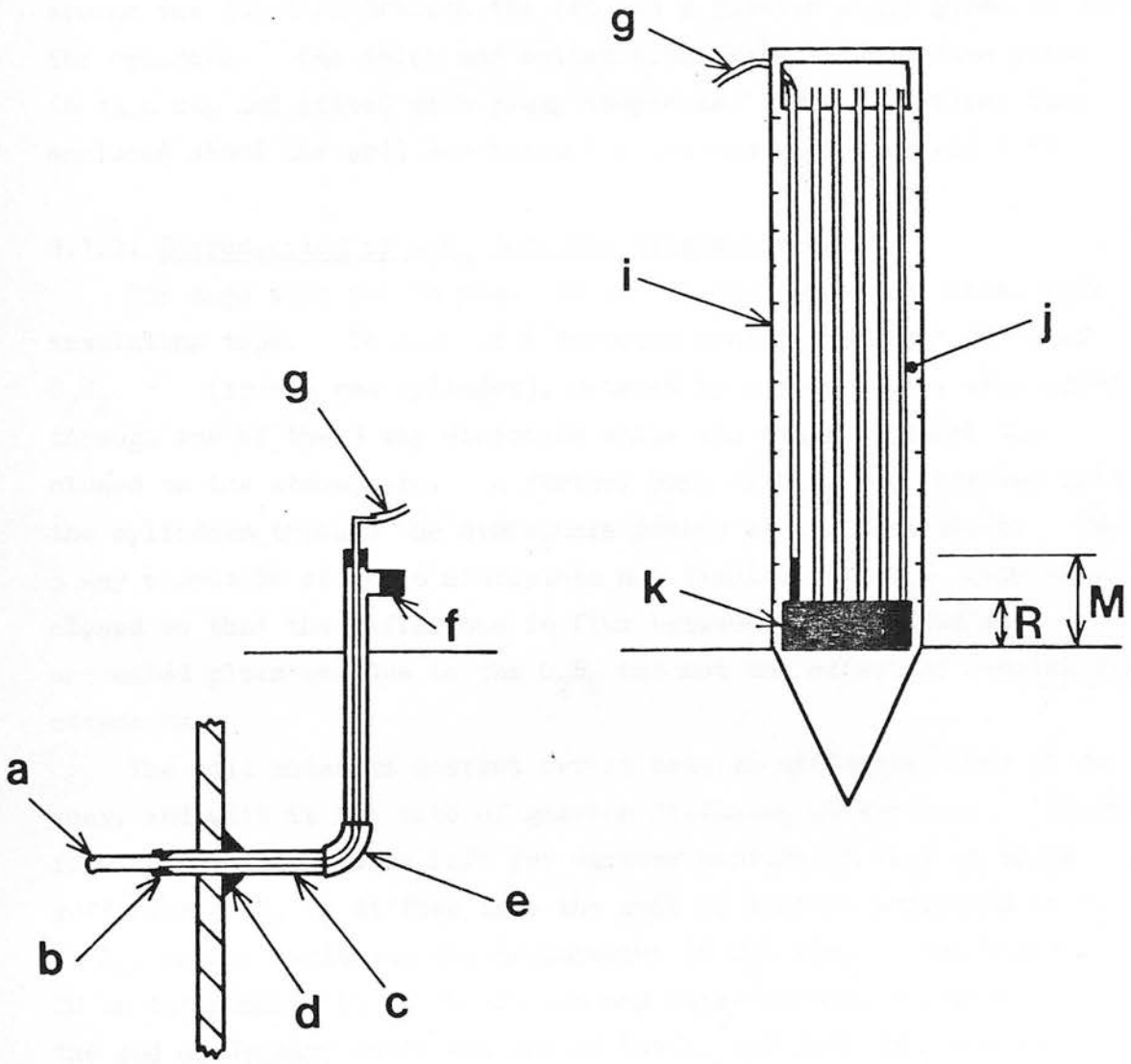


Fig. 8.3. Soil atmosphere sampler



a - porous pot, b - araldite seal painted with butumenous paint, c - rigid tube, d - plastic sealant, e - angled connector, f - side arm g - flexible tubing, i - wooden post, j - perspex plate screwed to post to hold tubes in place, k - mercury reservoir

Fig. 8.4. Tensiometer

respectively. The tensiometers were constructed as in Fig. 8.4 but without the angled connector, so that the porous pot was vertical in the soil. They were installed by augering a hole to the required depth, placing some loamy soil in the hole, pushing the tensiometer firmly into the soil and then packing the hole with more soil.

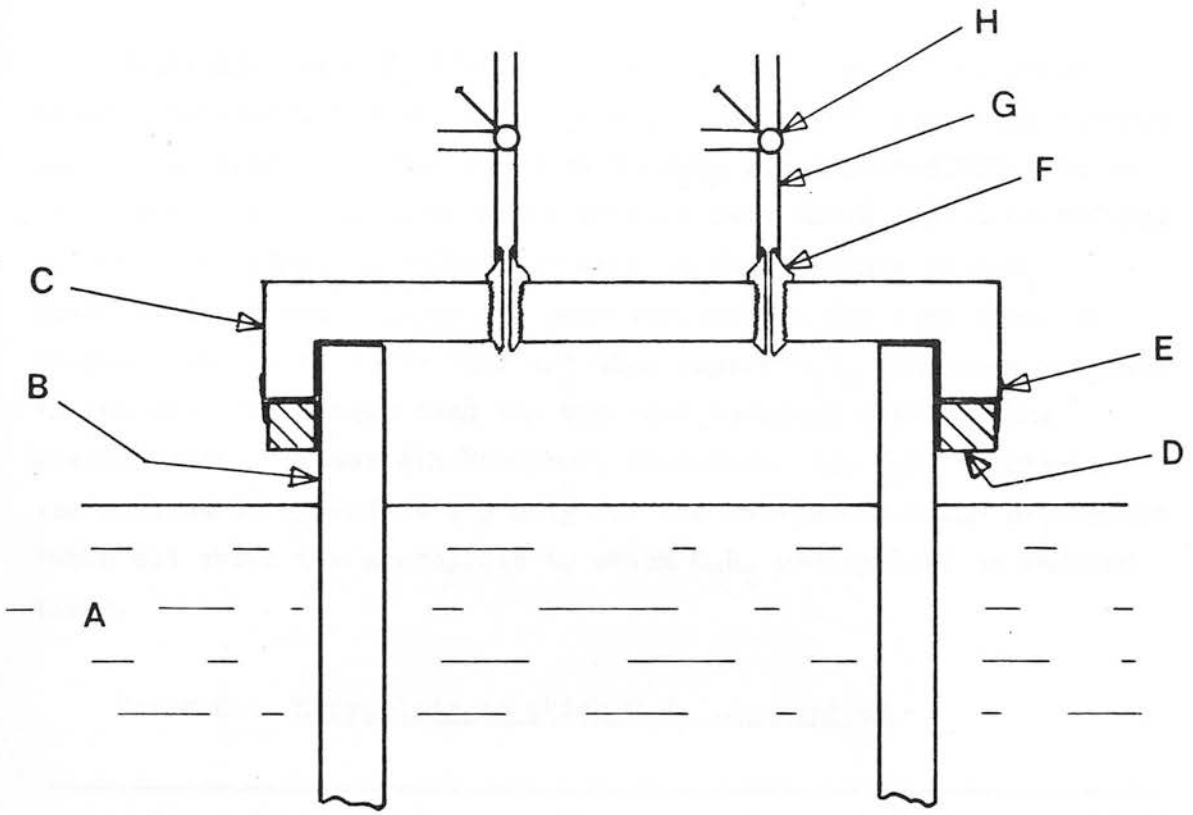
When all cylinders, soil atmosphere probes and tensiometers had been installed, the trench and the excavated areas around each cylinder were filled in.

Each cylinder could be covered with a well-fitting PVC cap (Fig. 8.5) sealed to the lysimeter with insulating tape wrapped around the junction between the cap and a plastic strip glued to the outside of the cylinder. Gas inlet and outlet tubes were screwed into holes in each cap and fitted with 3-way stopcocks. The air volume thus enclosed above the soil and below the cap was approximately 1.5l.

8.1.2. Introduction of C_2H_2 into the Microplots

The caps were put in place on all 12 cylinders and sealed with insulating tape. To each of 6 enclosed microplots about 400ml of C_2H_2 (from a gas cylinder), metered by a flow meter, were added through one of the 3 way stopcocks while the other stopcock was closed to the atmosphere. A further 50ml of C_2H_2 was injected into the cylinders through the atmosphere probes at the 30cm depth. The 3 way stopcocks of the 6 microplots not treated with C_2H_2 were also closed so that the difference in flux between C_2H_2-treated and untreated plots was due to the C_2H_2 and not the effect of closing the stopcocks.

The soil moisture content varied between different times of the year, and with it the rate of gaseous diffusion in the soil. Therefore the cylinders were left for various periods of time to allow sufficient C_2H_2 to diffuse into the soil to inhibit reduction of N_2O to N_2, before beginning the measurement of N_2O flux. The times were 2h in the summer, 5h in the autumn and early winter, overnight from the end of January until the end of March, and 3-5h from the beginning of April. The resulting mean C_2H_2 concentrations measured at the 0, 5, 10, 20, 30 and 40cm depth were 0.07, 0.04, 0.03, 0.01, 0.13 and 0.01ml ml^{-1} respectively.



A - soil, B - PVC cylinder, C - PVC lid, D - plastic strip,
E - insulating tape, F - threaded brass tube, G - plastic tubing
H - 3 way stopcock

Fig. 8.5. Arrangement for sealing the microplots

Initially the C_2H_2 treatment was alternated every few weeks between cylinders 1-3 and 10-12, and cylinders 4-9, since experiments had shown that prolonged exposure to C_2H_2 can affect nitrification (see Section 6.2) and some other workers have shown that denitrifying bacteria can adapt to reduce N_2O even in the presence of C_2H_2. However, after this regime had been implemented for some time, it became clear that at the 30cm and 40cm depths C_2H_2 sometimes remained in the soil for longer than the one week interval between flux measurements. After 4th November, therefore, the C_2H_2 treatment was applied to cylinders 4-9 only for the remainder of the experiment. Table 8.1 shows the microplots to which C_2H_2 was applied at various times.

Table 8.1. Microplots to which C_2H_2 was applied

Dates (inclusive)	Microplots to which C_2H_2 was applied
28.4.81. - 12.5.81	4-9
21.5.81. - 10.6.81.	1-3 and 10-12
2.7.81. - 21.7.81.	4-9
23.9.81. - 28.10.81.	1-3 and 10-12
4.11.81. - 26.5.82.	4-9

8.1.3. Procedure for Measuring Fluxes and Gas Concentration in the Soil Profile

The method adopted involved the use of an electric pump driven by a 12V car battery to draw a current of air (ca 150ml min^{-1}) through the airspace between the cap and the soil surface within each cylinder, and then, via a flow meter, through a series of traps containing magnesium perchlorate, soda lime and molecular sieve (m.s.) 5A (5×10^{-10} m) to remove water, CO_2 and N_2O respectively. The traps were made from lengths of PVC tubing. The m.s. was from Brentag, UK, and in the form of pellets of 1.6mm diameter. A single pump was connected to all 12 cylinders, and a set of traps acting as control, via a manifold. The arrangement is illustrated in

Plates 8.1-8.3 and Fig. 8.6.

An inlet tube about 0.5m long was connected to the inlet stopcock of each cylinder not treated with C_2H_2. Inlets to those cylinders treated with C_2H_2 were connected via a manifold to a Y piece, one arm of which was open to the air, while a small flow of C_2H_2 (10ml min^{-1}) passed into the other (Fig. 8.6). This arrangement was sufficient to maintain a concentration of 0.01-0.03 ml ml^{-1} C_2H_2 in the inlet air supply, thus ensuring that the C_2H_2 concentration in the soil was also maintained.

Operating Sequence

After the caps had been sealed in place for the appropriate length of time, gas samples were taken by syringe from all depths, to determine concentrations of O_2, N_2, CO_2, C_2H_2 and N_2O (Appendices 2 and 3). The apparatus was then set up as shown in Fig. 8.6, except that the m.s. traps were removed from the gas absorption train, and the pump was switched on for 10 min. to remove any N_2O which had accumulated in the headspace. The m.s. traps were then connected, and the pump switched on again for 1h. As a control the N_2O content of the ambient air was simultaneously measured by drawing an equal flow of air to that passing through each cylinder headspace directly through a gas absorption train.

In the laboratory the m.s. was tipped into 500ml volumetric flasks (of accurately known volume), fitted with drechsel heads, well greased with Apiezon grease, in which the central tubes had been replaced by silicone septums, and the side arms of which were connected to 3 way stopcocks (Fig. 6.3). Each flask was evacuated by connecting a pump to the 3 way stopcock, 40ml water was added by syringe through the silicone septum and the flask was well shaken. The water released absorbed N_2O (and also C_2H_2) from the m.s. (Dowdell and Crees, 1974; Ryden et al., 1978; Guthrie and Duxbury, 1978) which was then determined by gas chromatography (Appendices 2 and 3B). Contrary to the findings of Ryden et al. (1978) an equilibration time was not found necessary prior to gas analysis. From the volume of N_2O released, the flux of N_2O from the soil was

Plate 8.1. Closed microplots, with sampling tubes from soil atmosphere probe in foreground

molecular sieve traps

magnesium perchlorate and soda lime traps

flowmeters

to manifold

to microplots

Plate 8.2. Traps for CO_2, H_2O and N_2O and flowmeters

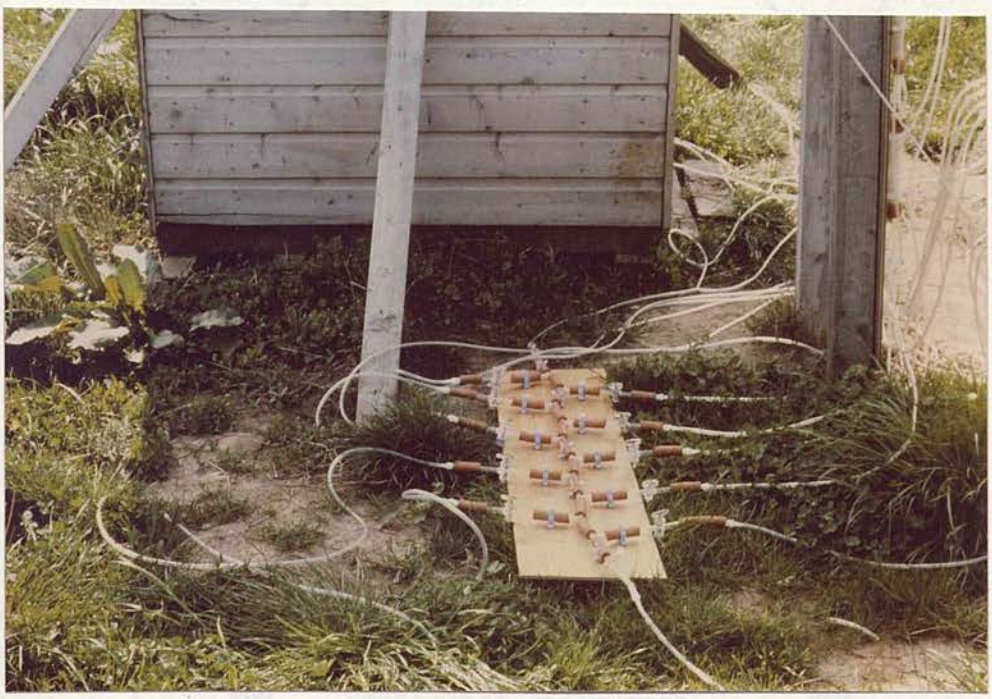


Plate 8.3. Manifold connecting the pump to the flowlines from the microplots

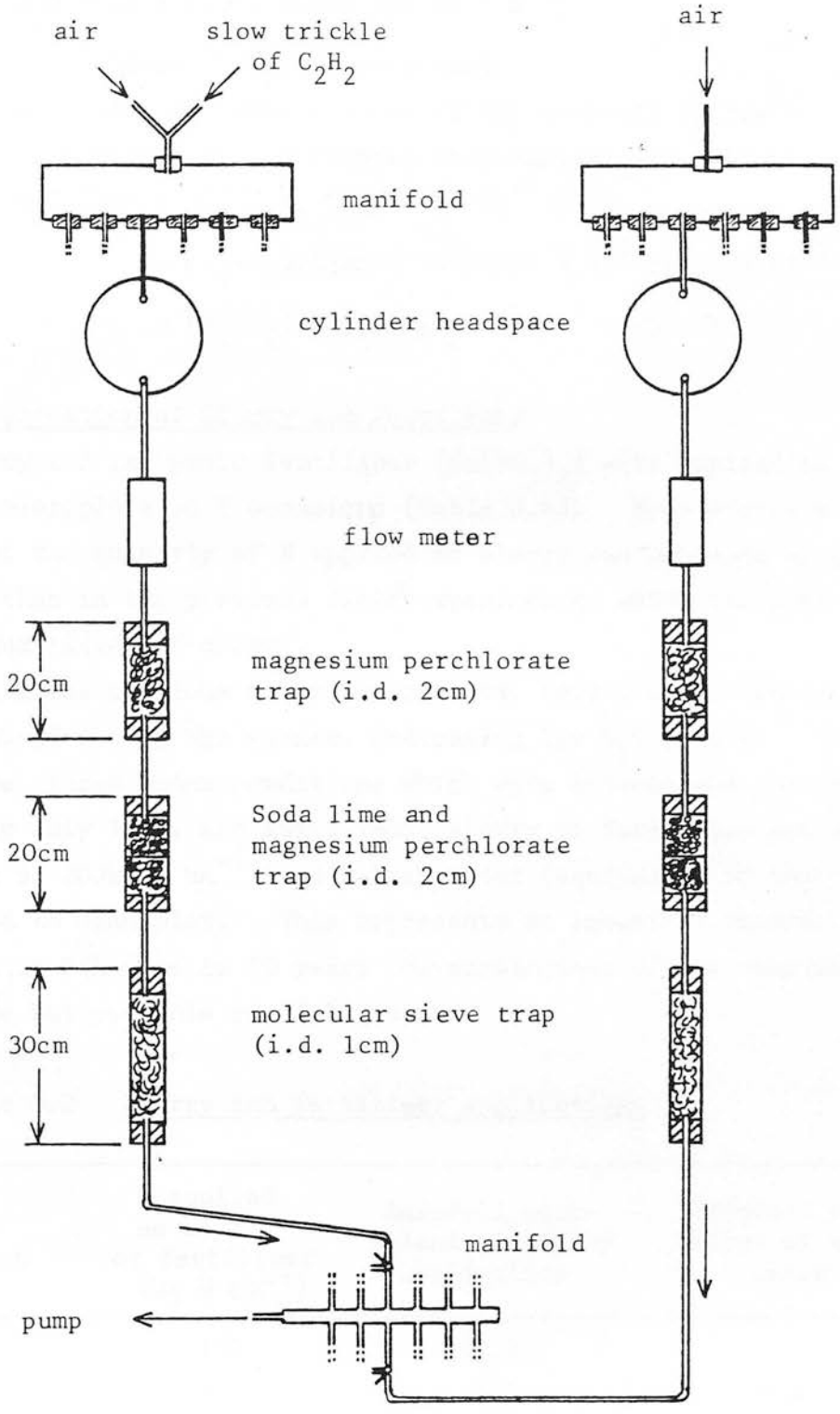


Fig. 8.6. Flow-line for trapping effluent N_2O

calculated:

$$N_2O \text{ flux} = (v_1 - v_2)/A \text{ (ml cm}^{-2} \text{ h}^{-1})$$

where v_1 = volume of N_2O trapped (ml)

A = area of cross section of the cylinder ($373 cm^2$)

v_2 = volume of N_2O trapped from ambient air control

$$N_2O \text{ flux} = 10^8 \times (v_1 - v_2)/A \text{ (ml ha}^{-1} \text{ h}^{-1})$$

$$= [(v_1 - v_2)/A] 28 \times 10^8 / 22.4 \times 10^3 \text{ (gN}_2\text{O-N ha}^{-1} \text{ h}^{-1})$$

$$= (v_1 - v_2) \times 335 \text{ (gN}_2\text{O-N ha}^{-1} \text{ h}^{-1}) \quad 8.2$$

8.1.4. Application of Slurry and Fertiliser

Slurry and inorganic fertiliser ($Ca(NO_3)_2$) were applied to the enclosed microplots on 5 occasions (Table 8.2). More accurate control of the quantity of N applied as slurry was achieved by prior analysis than in the previous field experiments, which required much greater quantities of slurry.

During the previous field experiments, very low N_2O concentrations were measured during the summer, indicating low N_2O fluxes. In order to measure fluxes under conditions which were extreme for the summer months, in July 1981, and April 1982, slurry or fertiliser was applied at a rate of 200kg N ha^{-1}, and 2 l of water (equivalent to 54mm rain) were added to each plot. This represents an amount of rainfall which falls within 24h once in 10 years (Meteorological Office records), i.e. an extreme but possible rainfall event.

Table 8.2 Slurry and fertiliser applications

Date of Application	N applied as slurry or fertiliser (kg N ha^{-1})	Rainfall equivalent of slurry application	Rainfall equivalent of added water
27.4.81	100	10.6	-
7.7.81	200	29.3	53.6
18.10.81	100	11.4	-
3.3.82	100	6.9	-
27.4.82	200	14.4	53.6

8.2. Soil Moisture Tension

The tensiometers installed in the cylinders were not satisfactory. Since the porous pots were horizontal, it was not always possible to flush air out by injecting water into the side arm and therefore the continuity of the water column between the porous pot and the mercury reservoir was broken. In spite of frequent attempts to flush out this air, by July it was apparent that few of the tensiometers were working properly. The initial readings of soil moisture tension are not presented because of the doubts about their accuracy.

New sets of tensiometers were therefore installed in November, as described in Section 8.1.1. At this time soil moisture tensions were low at all depths, and remained low until the end of March, 1982 in the surface soil, mid-April at 20 and 30cm and the end of April at 40cm (Fig. 8.7).

8.3. Temperature and Rainfall

Temperature and rainfall data (Table 8.3 and Fig. 8.8) are from the meteorological records of the Bush Estate, Penicuik.

Rainfall was particularly high in September and October and again in January (Table 8.3).

The soil temperature data (Fig. 8.8) illustrate the exceptionally cold winter of 1981/82. From mid December until 20th January, the surface soil was frozen except during the first week in January when there was a slight thaw. At this time the overlying snow began to melt and the surface of the ground was wet rather than frozen, although the temperature at the 10cm depth did not rise above $0^{\circ}C$.

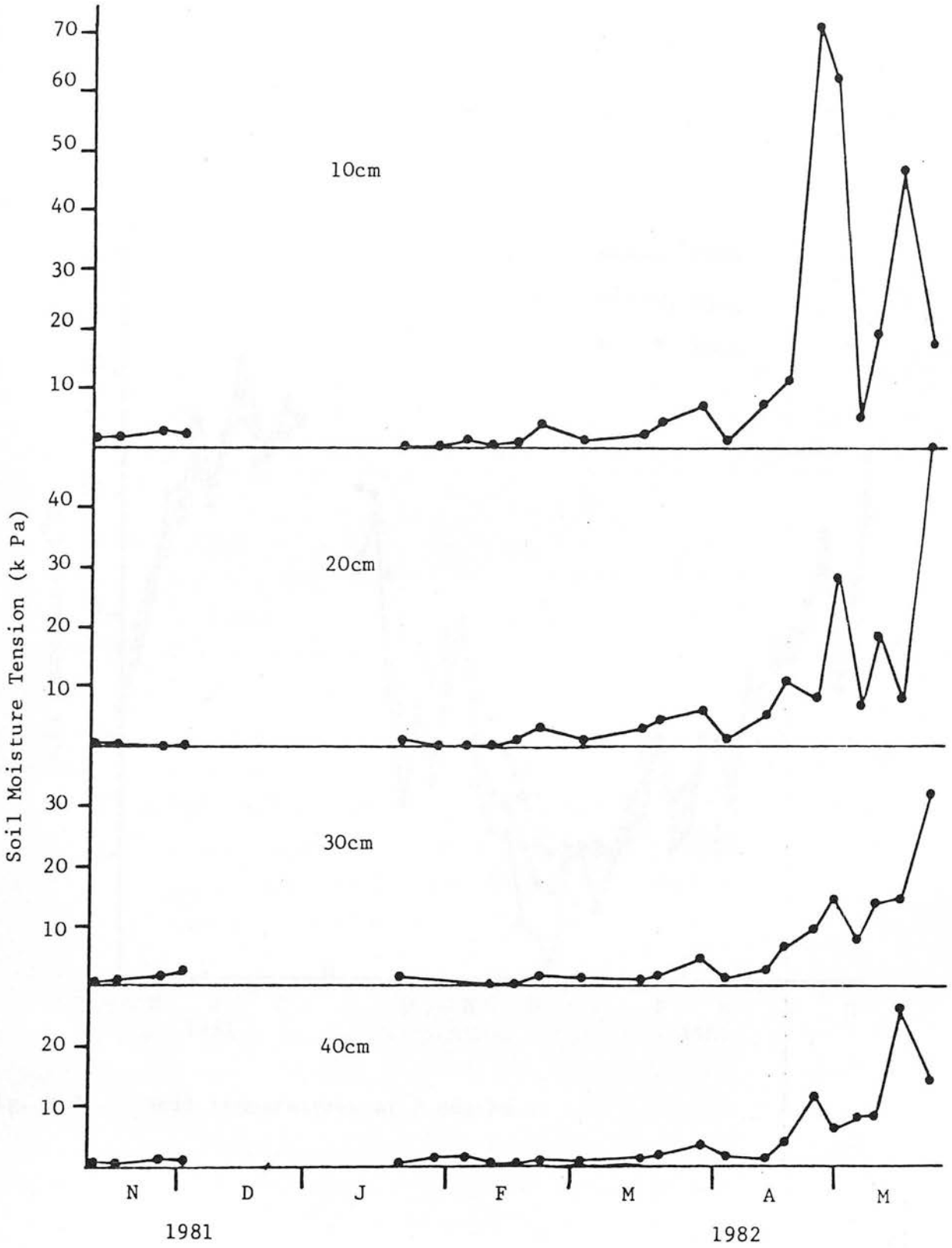


Fig. 8.7. Soil moisture tensions

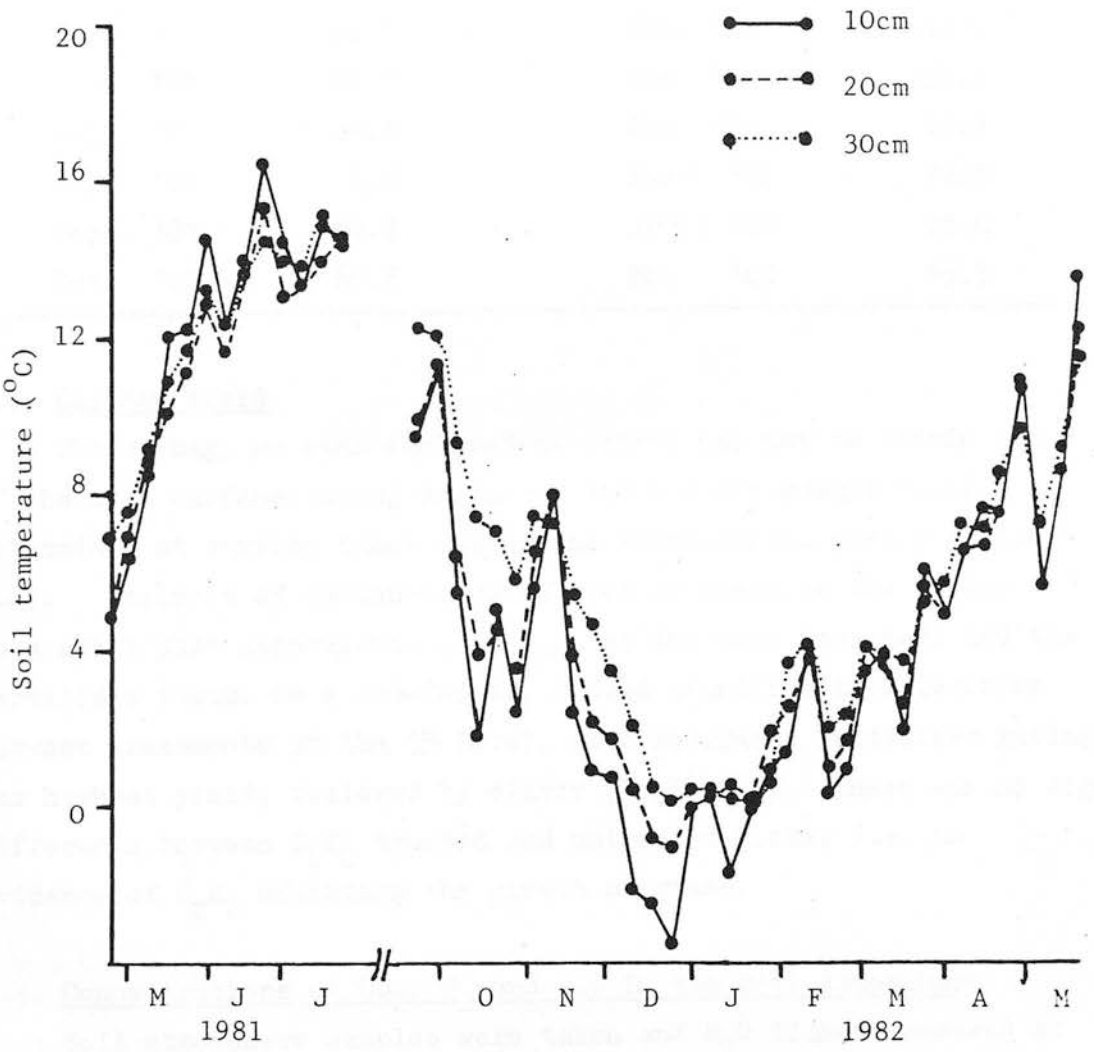


Fig. 8.8. Soil temperatures at 3 depths

Table 8.3. Monthly Rainfall

Month	Rainfall(mm)	Month	Rainfall(mm)
April '81	17.8	Nov. '81	93.0
May '81	46.5	Dec. '81	47.0
June '81	64.9	Jan. '82	136.3
July '81	69.1	Feb. '82	40.7
Aug. '81	7.3	March '82	72.8
Sept. '81	117.4	April '82	25.6
Oct. '81	169.3	May '82	63.5

8.4. Herbage Yield

The herbage on each enclosed microplot was cut to within 1cm of the soil surface, using scissors, and the dry weight yield determined at various times during the experimental period (Table 8.4). Analysis of variance, calculated by treating the design as a split plot experiment, with C_2H_2 as the main treatment and the fertiliser regime as a sub-factor, showed significant differences between treatments at the 5% level, with inorganic fertiliser giving the highest yield, followed by slurry (Table 8.5). There was no significant difference between C_2H_2 treated and untreated plots, i.e. no evidence of C_2H_2 affecting the growth of grass.

8.5. Concentrations of CO_2, O_2 and N_2O in the Soil Atmosphere

Soil atmosphere samples were taken and N_2O fluxes measured at approximately weekly intervals during the experimental period (more frequently immediately following an application of slurry and fertiliser), except from 20th July to 23rd September when no measurements were made. From mid December until 20th January no soil atmosphere samples could be taken because the ground was frozen.

8.5.1. Frequency Distributions for CO_2 and O_2

The frequency distribution of the untransformed CO_2 data (Fig. 8.9) was positively skewed and shows that, in plots treated

Table 8.4. Dry weight yields of microplots

Treatment	C_2H_2 treatment[a]	Microplot No.	Dry Weight yield(g)
Control	$+C_2H_2$	4	36.9
		9	28.1
	$-C_2H_2$	3	36.9
		12	26.4
Slurry	$+C_2H_2$	5	58.3
		8	66.6
	$-C_2H_2$	1	56.1
		11	48.4
Inorganic Fertiliser	$+C_2H_2$	6	63.8
		7	65.2
	$-C_2H_2$	2	69.1
		10	74.1

(a) During 9 weeks of the experimental period Microplots 1-3 and 10-12 were treated with C_2H_2 (see Table 8.1).

Table 8.5. Analysis of variance for herbage yield

Source	df	ss	ms	F
C_2H_2 treatment	1	15.61	15.61	0.36
Error	2	87.52	43.76	
Total	3	103.13		
N Treatments	2	2730	1365	74.3***
(Control different from others	1	2501	2501	136.1***)
(Slurry different from fertiliser	1	229	229	12.5*)
N treatment x C_2H_2 treatment	2	150	75	4.1
Error	4	73.5	18	
Total	11	3056.6		

Note: Partitioning of treatment sum of squares was as described by Pearce (1965).

with C_2H_2, CO_2 concentrations were slightly higher than in untreated plots. The data were transformed to an approximately normal distribution (Fig. 8.10) by the transformation used previously (Equation 2.2).

Mean concentrations of CO_2 were higher under the slurried treatment than the control and fertilised treatments and were higher in plots treated with C_2H_2 (Table 8.6). A paired t-test showed that the difference between CO_2 concentrations in the C_2H_2- and non-C_2H_2-treated plots was significant at the 0.1% level under the control and slurry treatments but was not significant under the fertilised treatment.

Increased CO_2 concentrations in C_2H_2 treated plots occurred mostly at the end of the experimental period but not at any specific depth (Fig.8.11).

Table 8.6. Mean CO_2 concentrations over all times and depths

C_2H_2 treatment	Mean CO_2 concentration					
	mean of transformed data			reverse transform of mean (ml ml $^{-1}$ x 10^2)		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised
$+C_2H_2$	0.68	0.83	0.65	0.98	1.30	0.90
$-C_2H_2$	0.61	0.74	0.62	0.84	1.09	0.86

The frequency distribution of the untransformed O_2 data was not normally distributed and was negatively skewed (Figs. 8.12 and 8.13). Median O_2 concentrations for the slurried, fertilised and control lysimeters were 0.186, 0.194 and 0.194 ml ml^{-1}, respectively. The frequency distribution of data with and without C_2H_2 (Fig. 8.12) showed that O_2 concentrations were lower in plots to which C_2H_2 had been applied.

The transformation used in the randomised block field experiment (Equation 3.1) gave an approximately normal distribution (Fig. 8.14).

Mean O_2 concentrations in the plots under the three treatments with and without C_2H_2 (Table 8.7) show that differences between

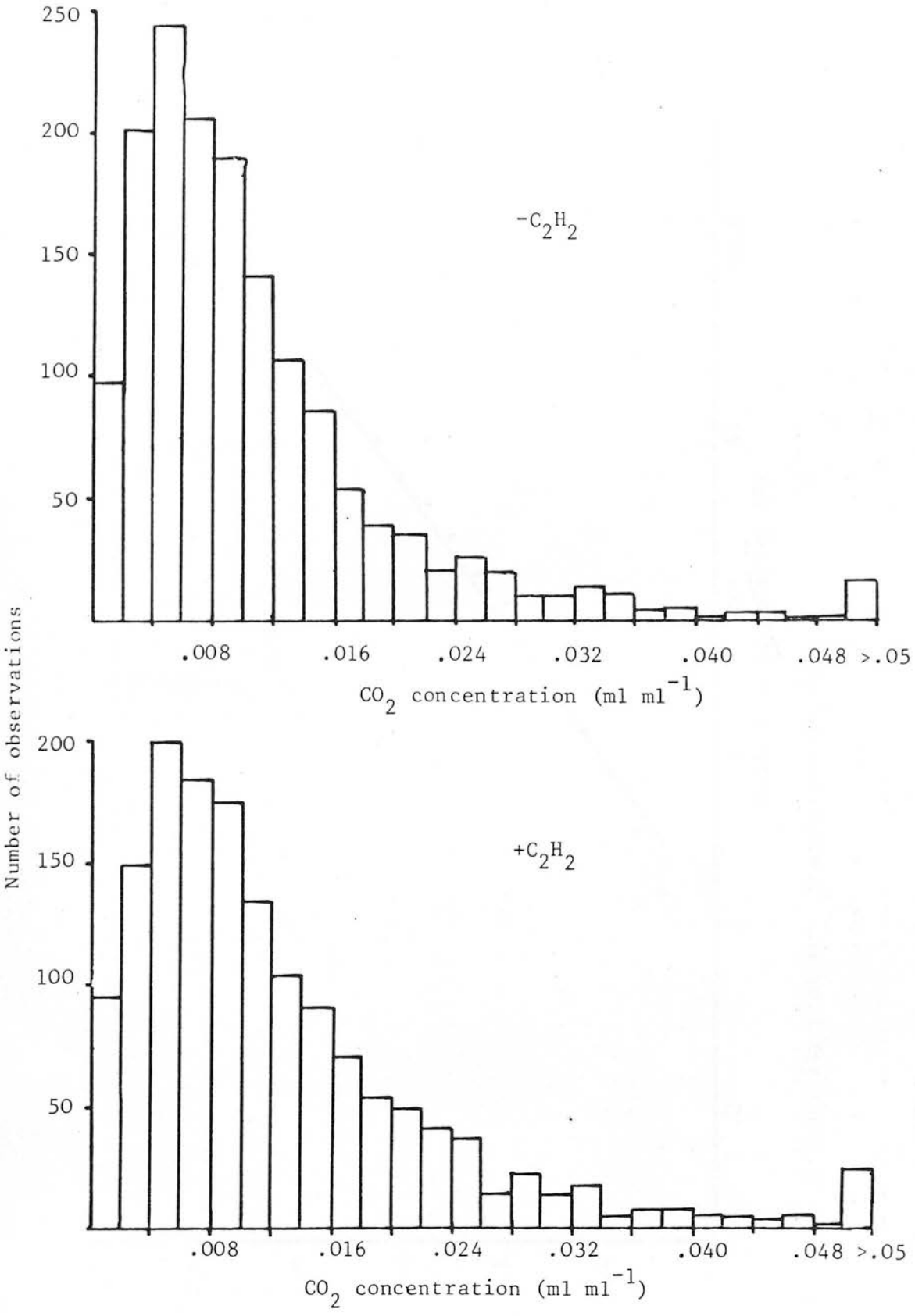


Fig. 8.9. Frequency distributions for CO_2 data in microplots with and without C_2H_2

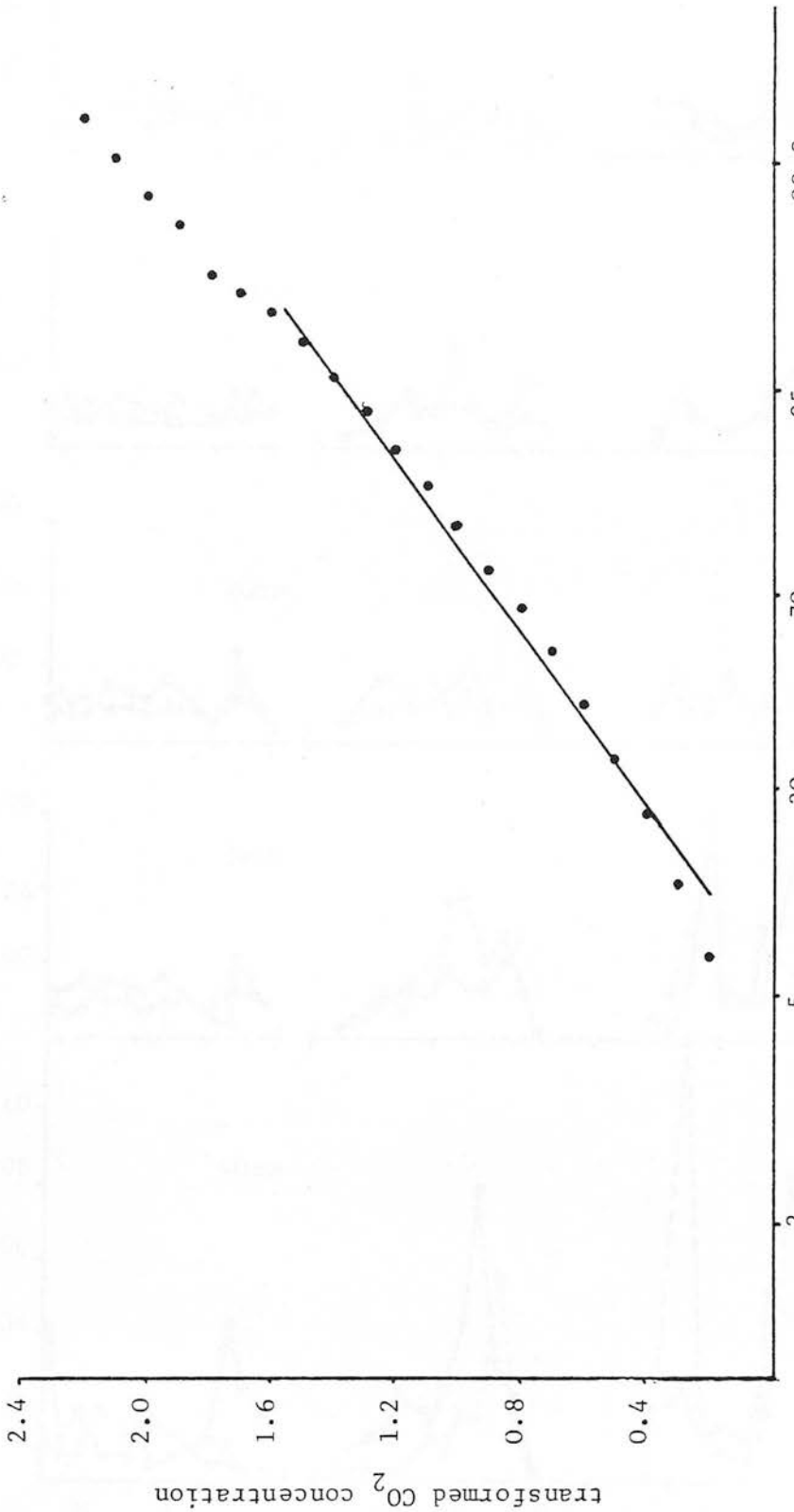


Fig. 8.10. Probability plot for transformed CO_2 data

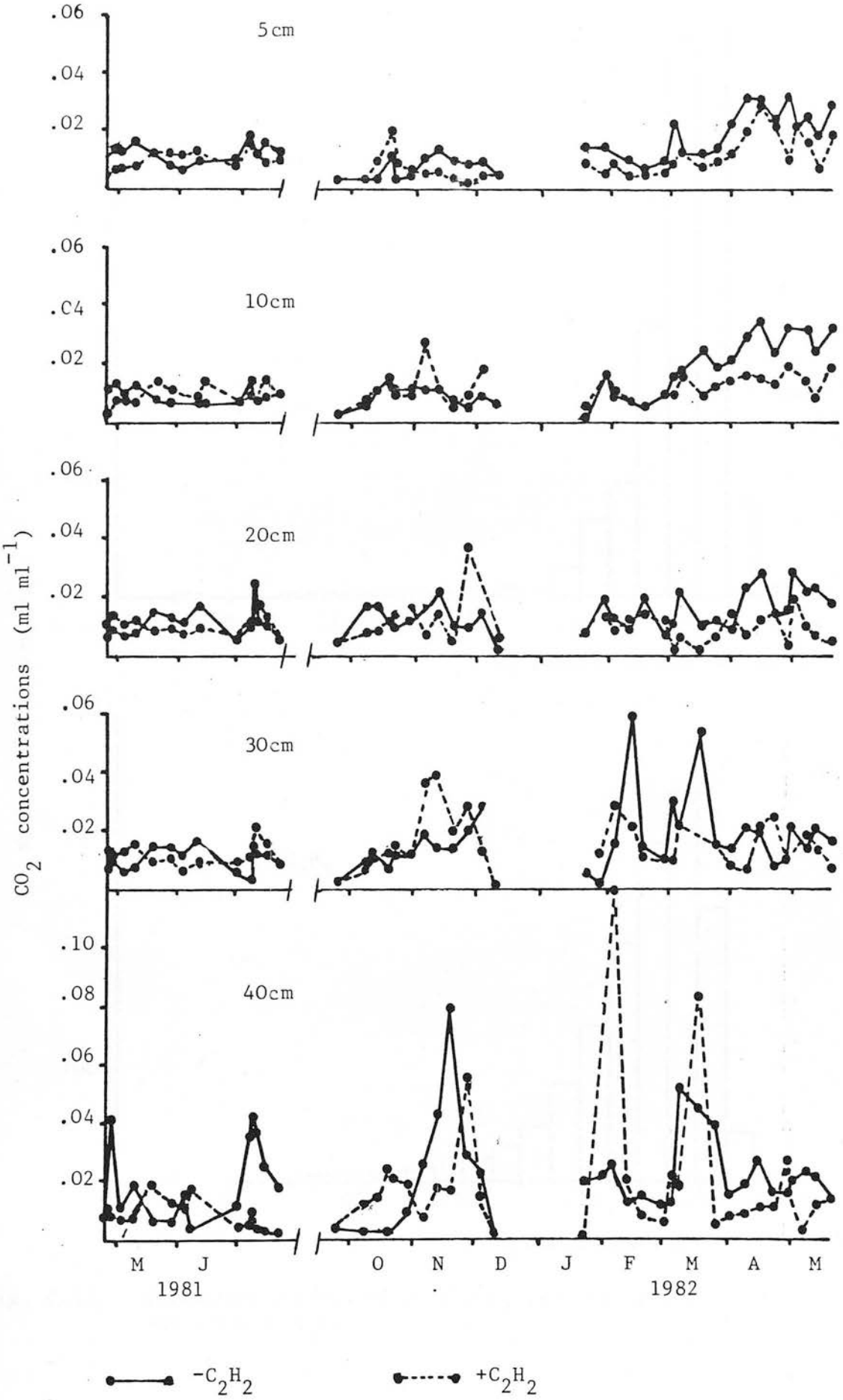


Fig. 8.11. Mean CO_2 concentrations with and without C_2H_2 in slurried microplots.

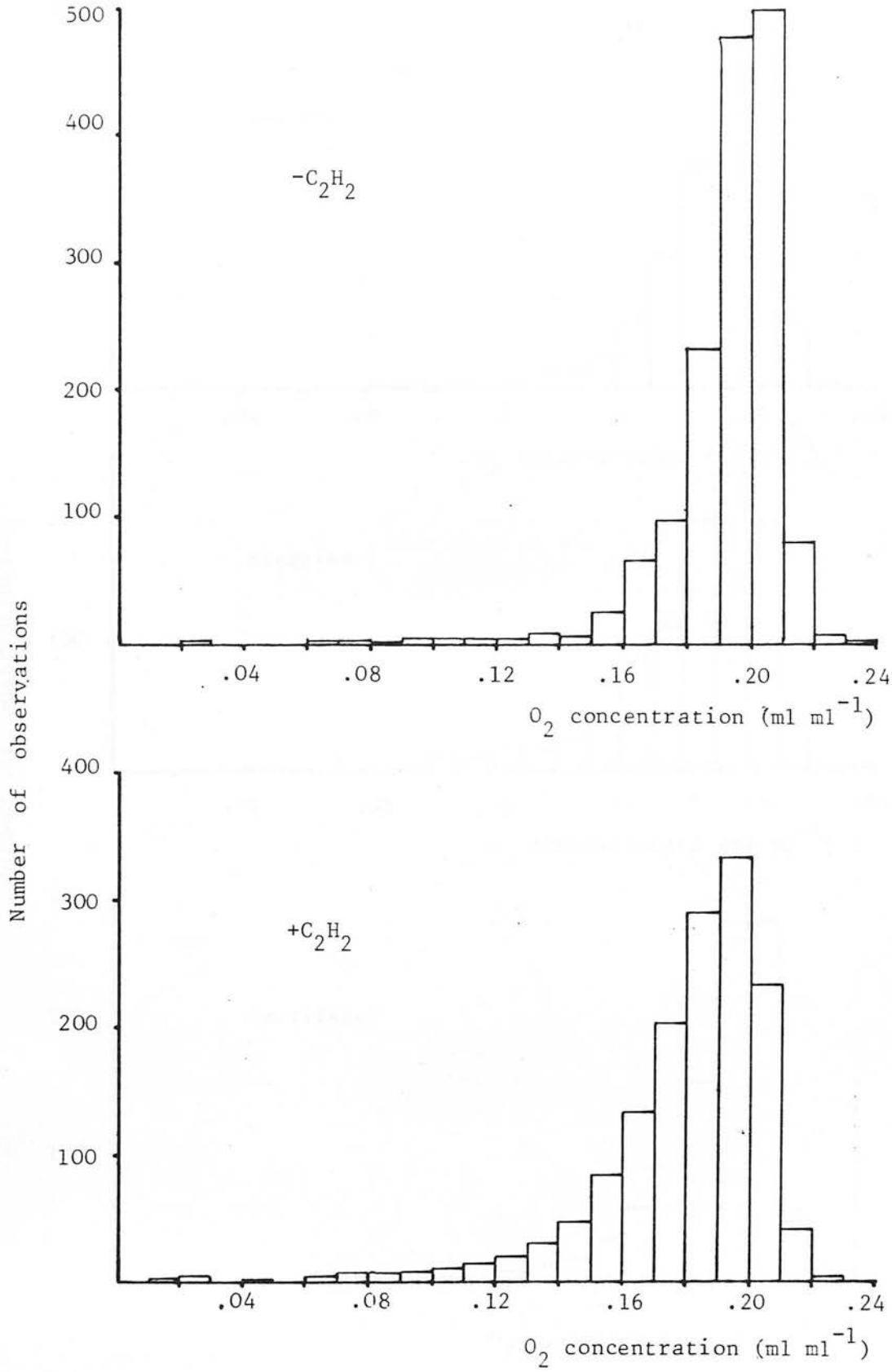


Fig. 8.12. Frequency distributions for O_2 data in microplots with and without C_2H_2

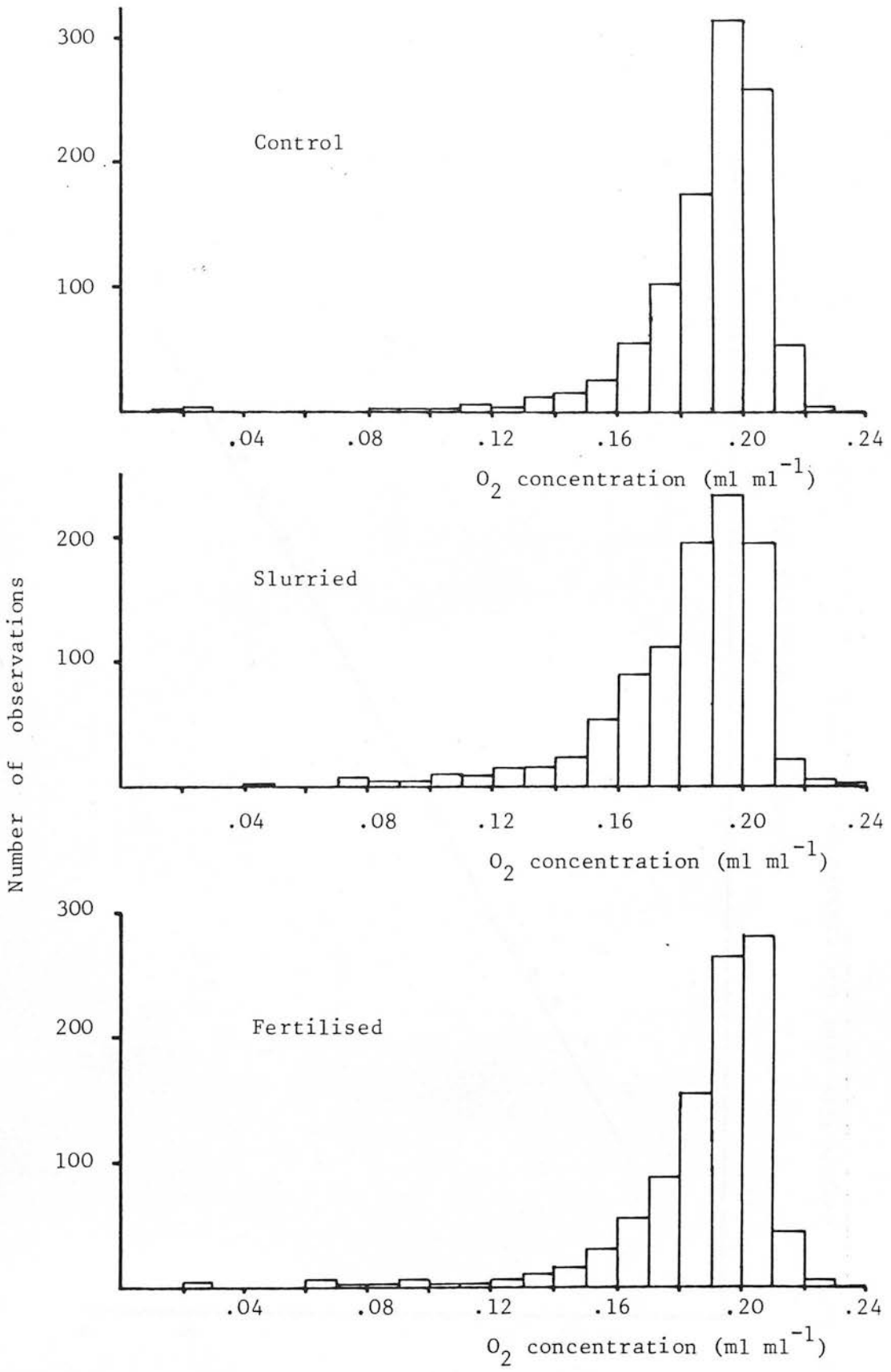


Fig. 8.13. Frequency distributions for O_2 data in control, slurried and fertilised microplots

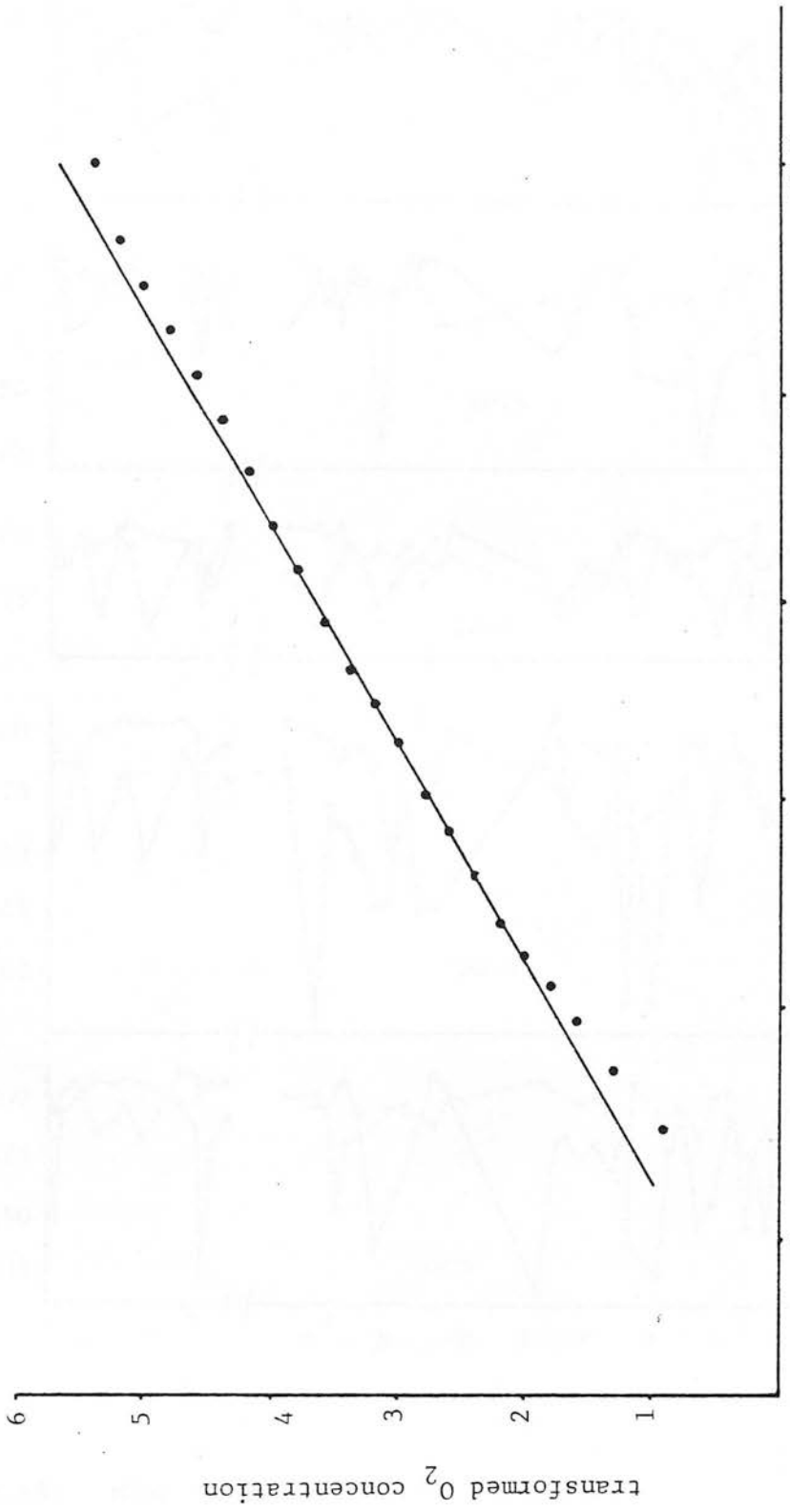


Fig. 8.14. Probability plot for transformed O_2 data

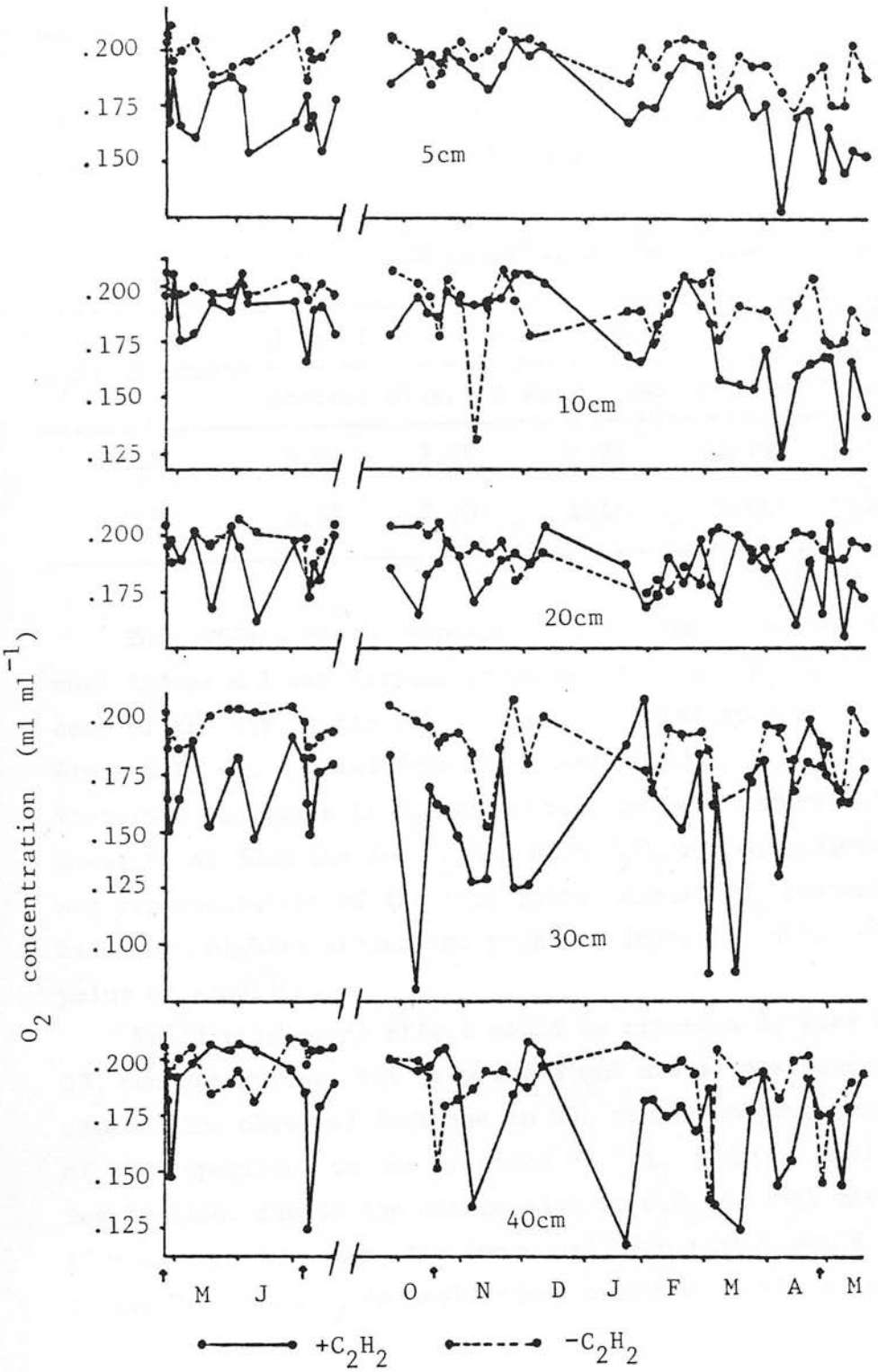


Fig. 8.15. Mean O_2 concentrations with and without C_2H_2 in slurried microplots (arrows indicate applications of slurry and fertiliser)

treatments were small but a paired t-test showed that the difference between C_2H_2-treated and untreated plots was significant at the 0.1% level for all treatments. The reduction in O_2 concentrations occurred at all depths and times but was particularly marked at 5 and 30cm where C_2H_2 concentrations were highest (Fig. 8.15).

Table 8.7. Mean O_2 Concentrations over all Times and Depths

C_2H_2 treatment	Mean of transformed O_2 data			Reverse transform of mean (ml ml^{-1})		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised
$+C_2H_2$	2.84	3.15	2.97	0.198	0.194	0.196
$-C_2H_2$	2.55	2.70	2.43	0.201	0.200	0.202

This effect can be explained by the fact that the presence of C_2H_2 must induce a lower partial pressure of O_2 and N_2 by displacement of some of the air in the soil, since the total pressure does not increase. Since C_2H_2 was applied from above and at 30cm, C_2H_2 concentrations (and therefore decreases in O_2 concentrations) were highest at these depths. However, at 30cm the low O_2 and high C_2H_2 concentrations observed were not representative of the 30cm plane, since C_2H_2 concentrations would have been highest around the point of injection which was also the point of sampling.

The displacement effect could be expected to give rise to lower CO_2 concentrations, but as pointed out above, the reverse was the case. The observed increase in CO_2 concentrations towards the end of the experiment in the presence of C_2H_2 could be explained by increased respiration, due to the consumption of C_2H_2 by soil microorganisms. If this were the case, the increased respiration would also contribute to the decreased O_2 concentrations observed in the presence of C_2H_2.

8.5.2. Frequency Distribution for N_2O

The frequency distributions for the untransformed N_2O data (Figs. 8.16 and 8.17) were positively skewed. Median values were 0.6, 0.7, and 0.9 x 10^{-6} ml ml^{-1} in the control, slurried and fertilised plots, respectively. Most of the very high N_2O concentrations recorded were from the fertilised plots where concentrations ranged up to 440 x 10^{-6} ml ml^{-1}. There was little apparent difference in the distribution of N_2O concentrations with and without C_2H_2 (Fig. 8.16).

The data were transformed to an approximately normal distribution (Fig. 8.18) by the transform used previously for N_2O (Equation 3.2).

The results show that, for the slurried and control plots, mean N_2O concentrations, calculated using the transformed data, were higher in the absence of C_2H_2 (Table 8.8). However where C_2H_2 was applied the frequency of high N_2O concentrations increased, e.g. in the slurried plots 4.3% and 2.5% of observed values were greater than 5 x 10^{-6} ml ml^{-1} in C_2H_2-treated and untreated plots respectively. A paired t-test showed that the effect of C_2H_2 or N_2O concentrations was significant at the 0.1, 1, and 5% level for the fertilised, slurried and control plots respectively. The unexpected decrease in N_2O concentrations in the presence of C_2H_2 for the control and slurried plots indicates that the effect of C_2H_2 was less than random differences between the micro-plots except when N_2O concentrations were high, in spite of the fact that O_2 concentrations were significantly lower in C_2H_2 treated plots. This provides some evidence that at higher rates of denitrification, e.g. when the soil is very wet, diffusion is slow and therefore N_2O is more likely to be reduced before reaching the soil surface.

Table 8.8. Mean N_2O concentrations over all times and depths

C_2H_2 treatment	Mean of transformed data			Reverse transform of mean (ml ml^{-1} x 10^{-6})		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised
$+C_2H_2$	3.28	3.34	5.08	0.47	0.48	1.11
$-C_2H_2$	3.51	3.66	4.44	0.51	0.55	0.86

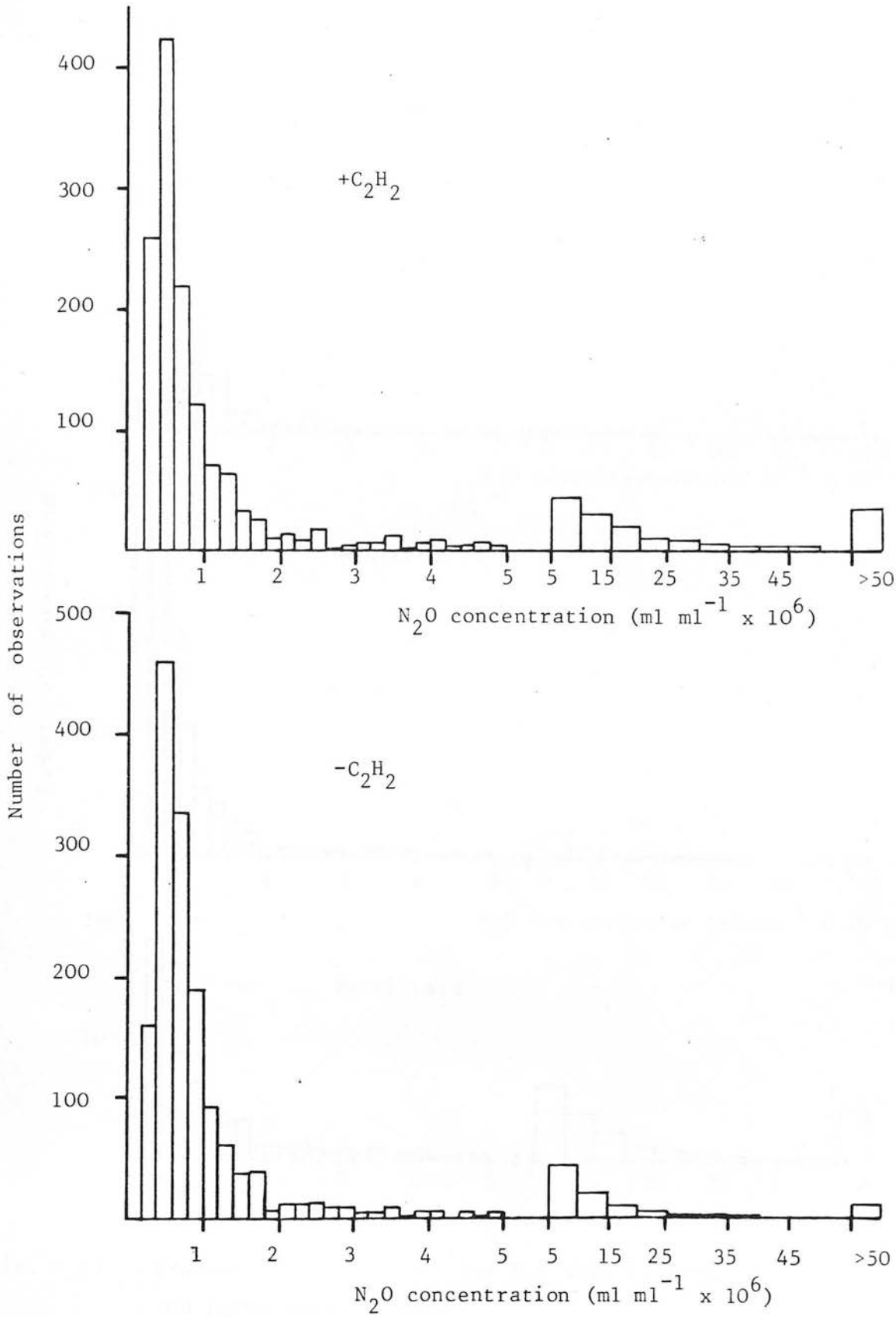


Fig. 8.16. Frequency distributions for N_2O data in microplots with and without C_2H_2

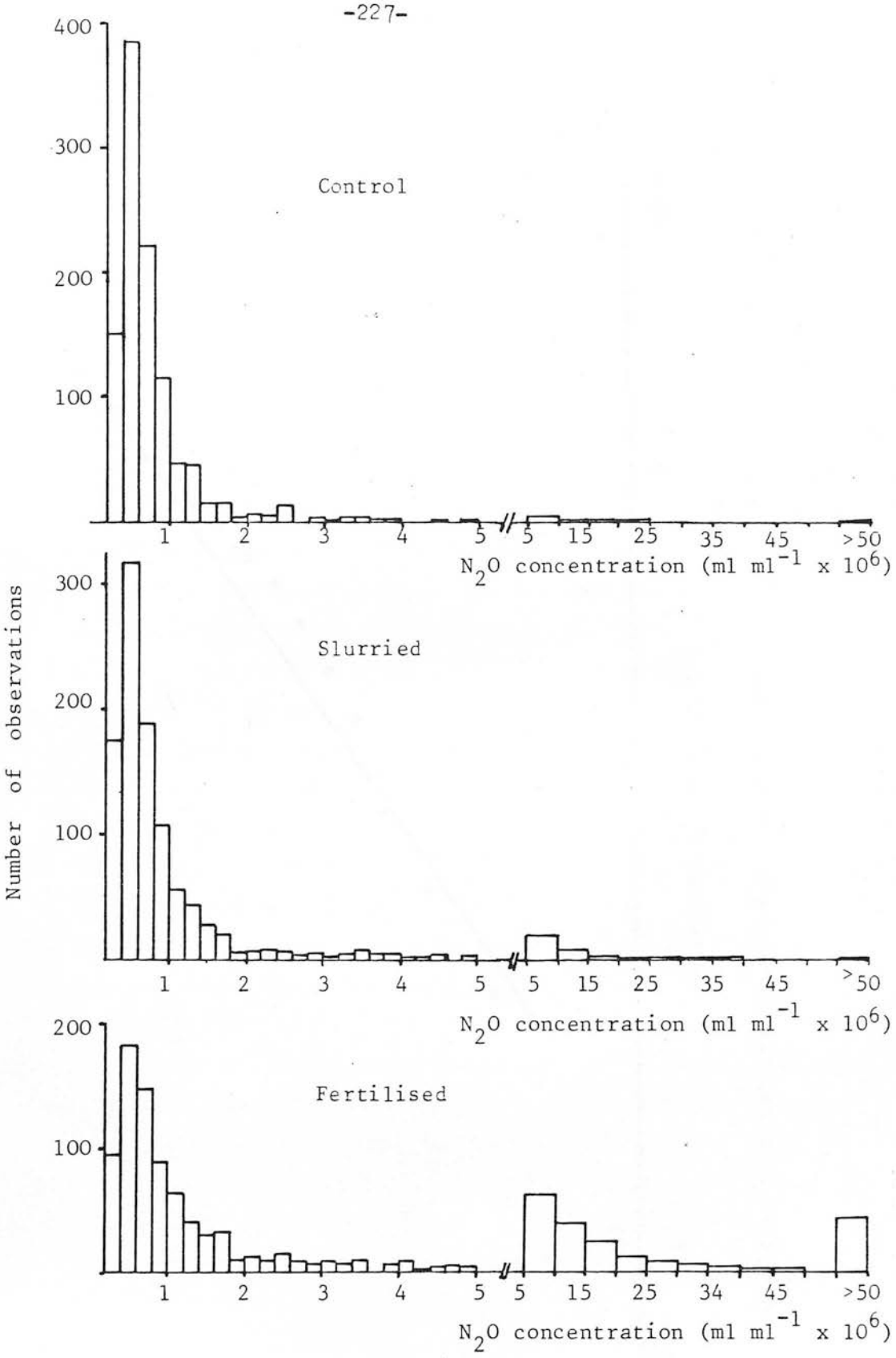


Fig. 8.17. Frequency distributions for N_2O data in control, slurried, and fertilised microplots

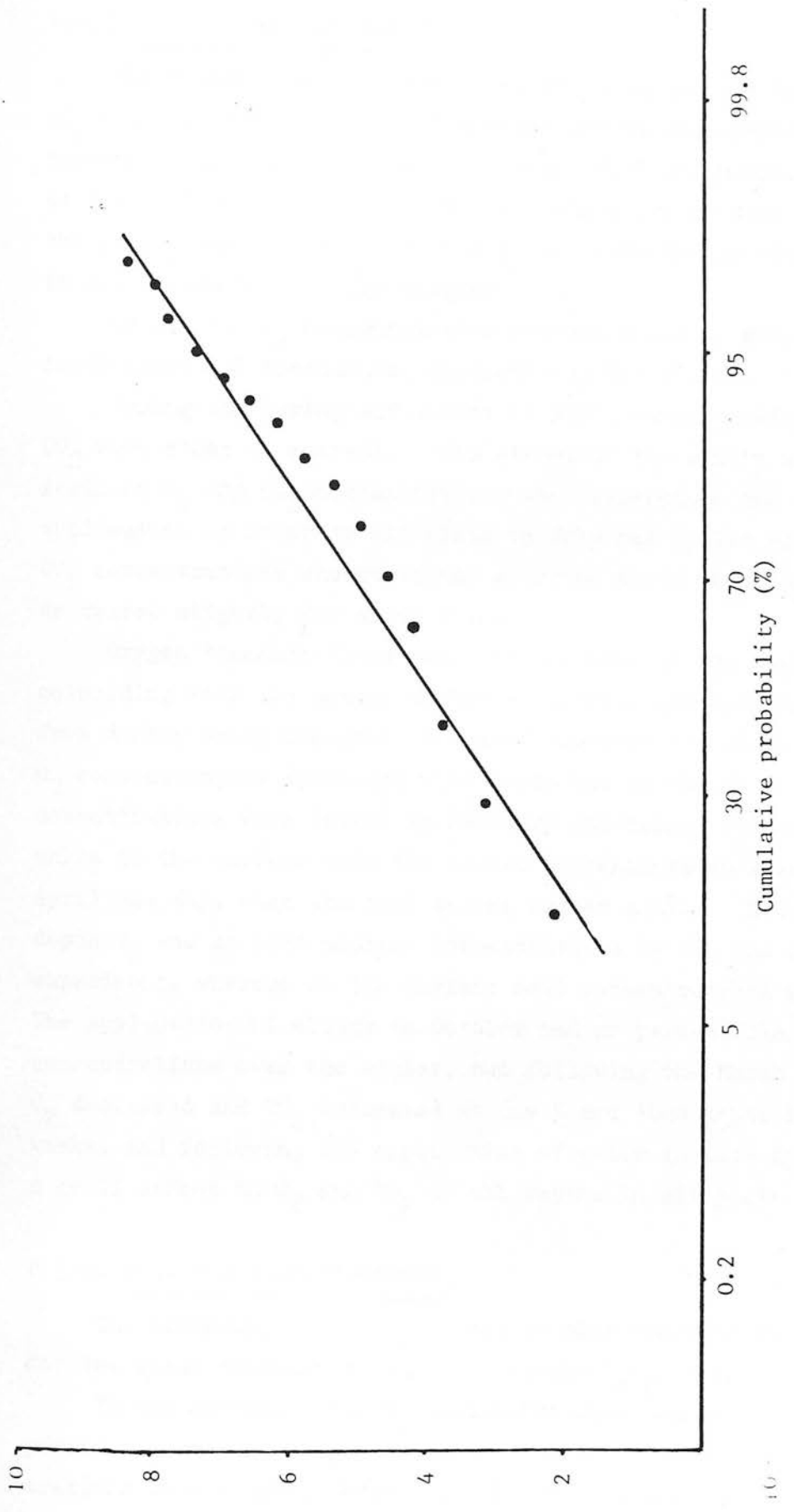


Fig. 8.18. Probability plot for transformed N_2O concentrations

8.5.3. Soil O_2 and CO_2 Concentrations

The transformed data were used to calculate the mean O_2 and CO_2 concentrations for each treatment and depth, combining data from treatments with and without C_2H_2 (Figs. 8.19 and 8.20). The long period in December and January where there are no data was because the ground was frozen (Section 8.3) and even during the slight thaw it was impossible to take samples.

Generally CO_2 concentrations increased and O_2 decreased with depth under all treatments, particularly below 20cm.

During the spring and summer of 1981, concentrations of O_2 and CO_2 were close to ambient. The effect of the slurry application in April on O_2 and CO_2 concentrations was discernible but small. The application of water to all plots in July had little effect on O_2 and CO_2 concentrations except in the slurried plots where O_2 concentrations decreased slightly for about 2 weeks.

Oxygen concentrations began to decrease at the beginning of October coinciding with the period of heavy rainfall (Section 8.3) and, apart from during early December, remained low over the winter. Generally O_2 concentrations decreased with depth but at the 30 and 40cm depth, concentrations were lowest in February and March, following the thaw, while in the surface soil the lowest concentrations were recorded in April and May, when the soil became warmer again. Below the 20cm depth O_2 was at near ambient concentrations by the end of the experiment, whereas in the surface soil concentrations were still low. The application of slurry in October had no perceptible effect on O_2 concentrations over the winter, but following the March application, O_2 decreased and CO_2 increased at the 5 and 10cm depth for several weeks, and following the application of water in late April there was a small effect on O_2 and CO_2 at all depths in all plots.

8.5.4. Soil N_2O Concentrations

The transformed data were used to plot the mean N_2O concentrations for the three treatments with and without C_2H_2 (Figs. 8.21 to 8.23).

In the control plots N_2O concentrations remained low over the entire period: there was no increase even in the winter and spring. Concentrations were slightly higher at 20 and 30cm than at other depths. The

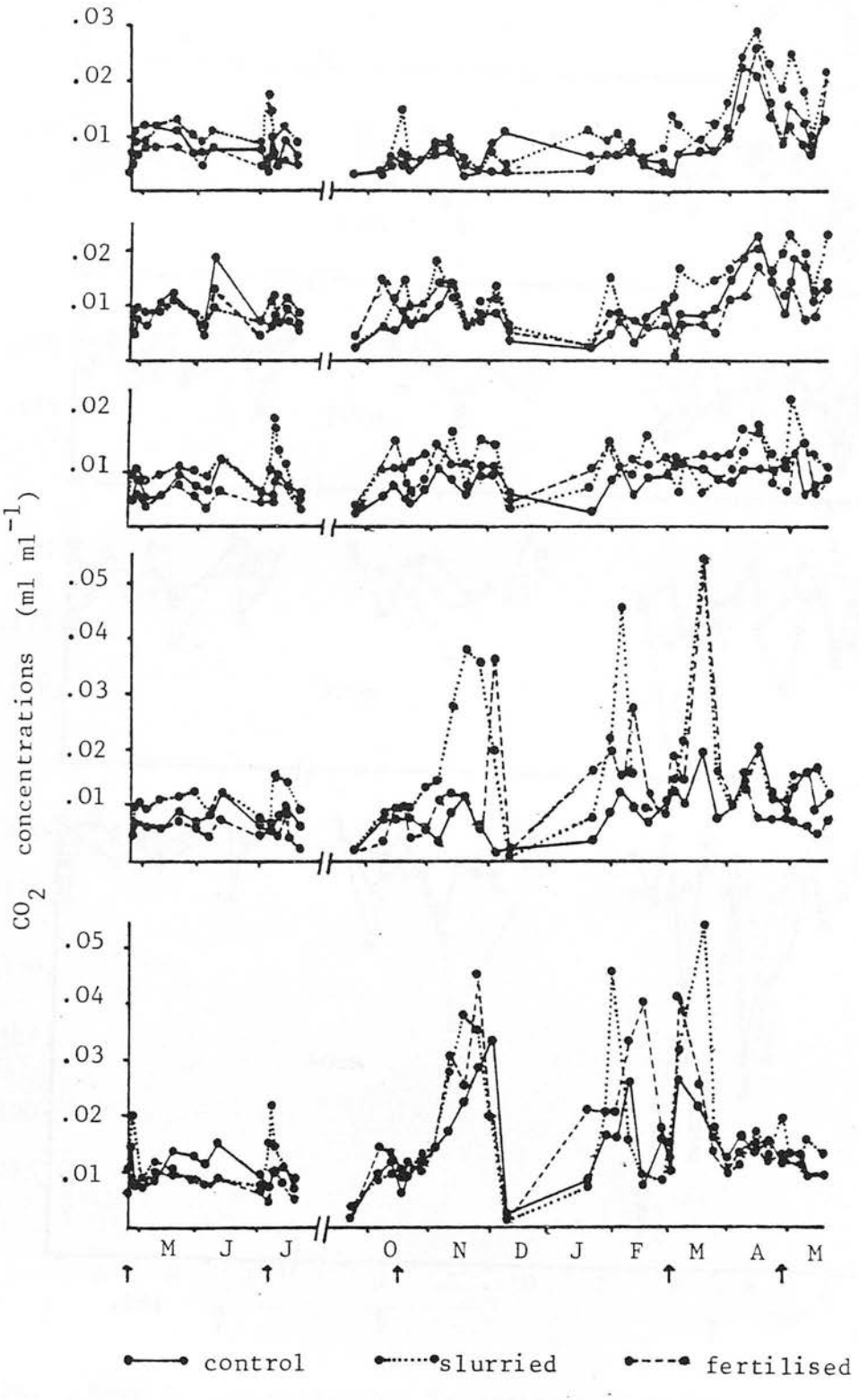


Fig. 8.19. Mean CO_2 concentrations in control, slurried and fertilised microplots (arrows indicate applications of slurry and fertiliser)

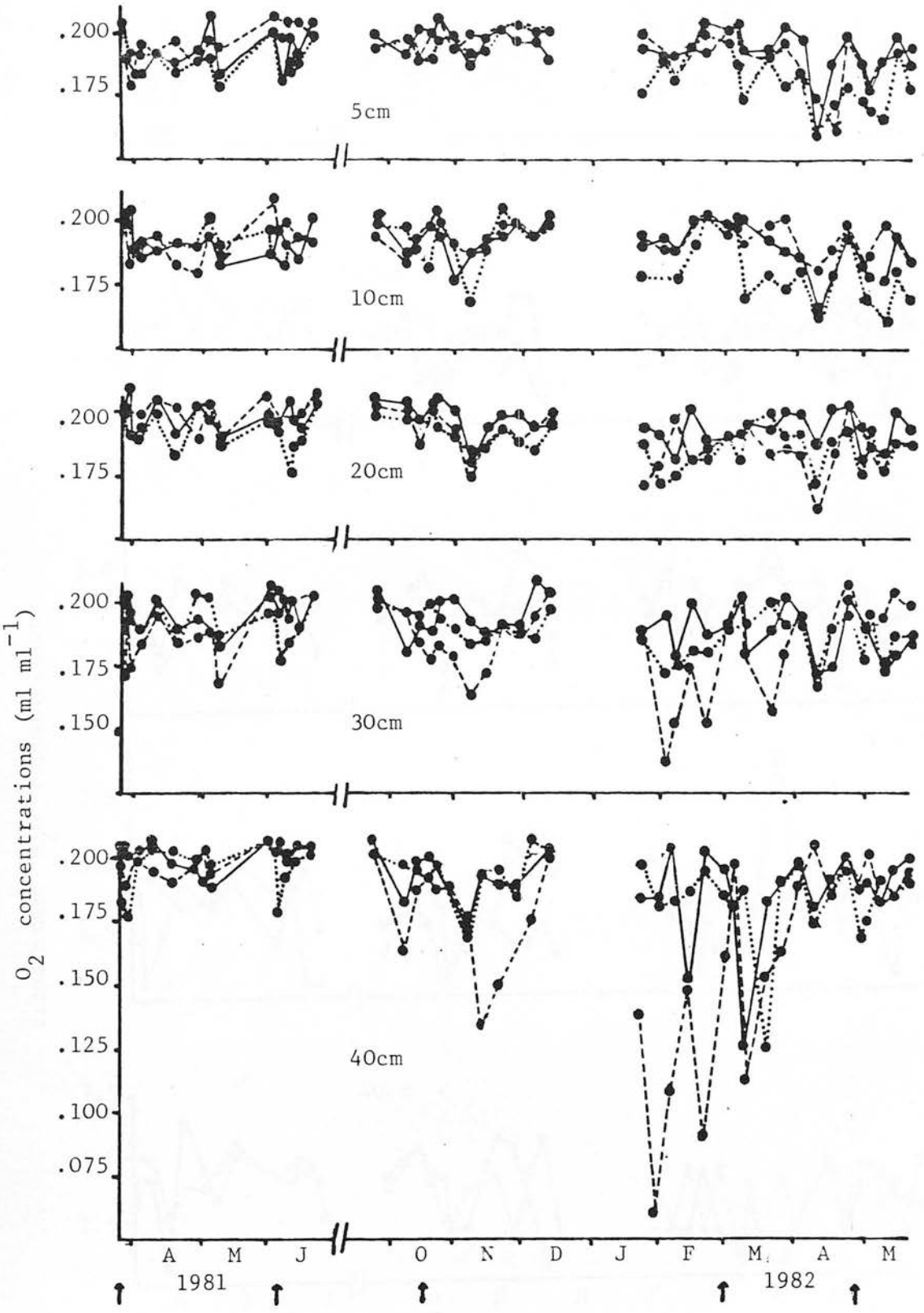


Fig. 8.20. Mean O_2 concentrations in control, slurried and fertilised microplots (arrows indicate applications of slurry and fertiliser)

control slurried fertilised

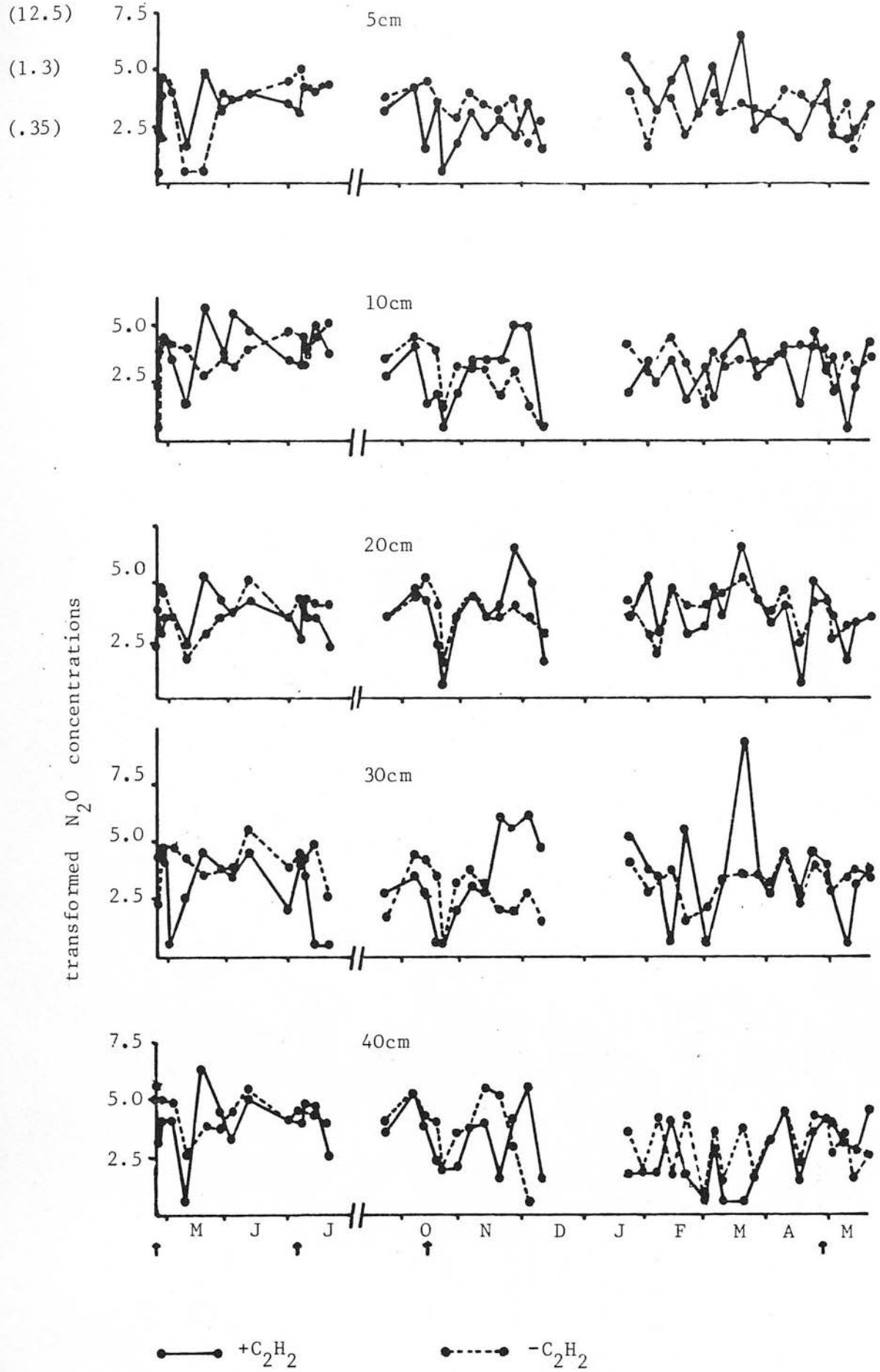


Fig. 8.21. Mean N_2O concentrations in control microplots with and without C_2H_2 (arrows indicate applications of slurry and fertiliser. Figures for N_2O given in brackets are the reverse transform of N_2O concentrations (ml ml^{-1} x 10^6).

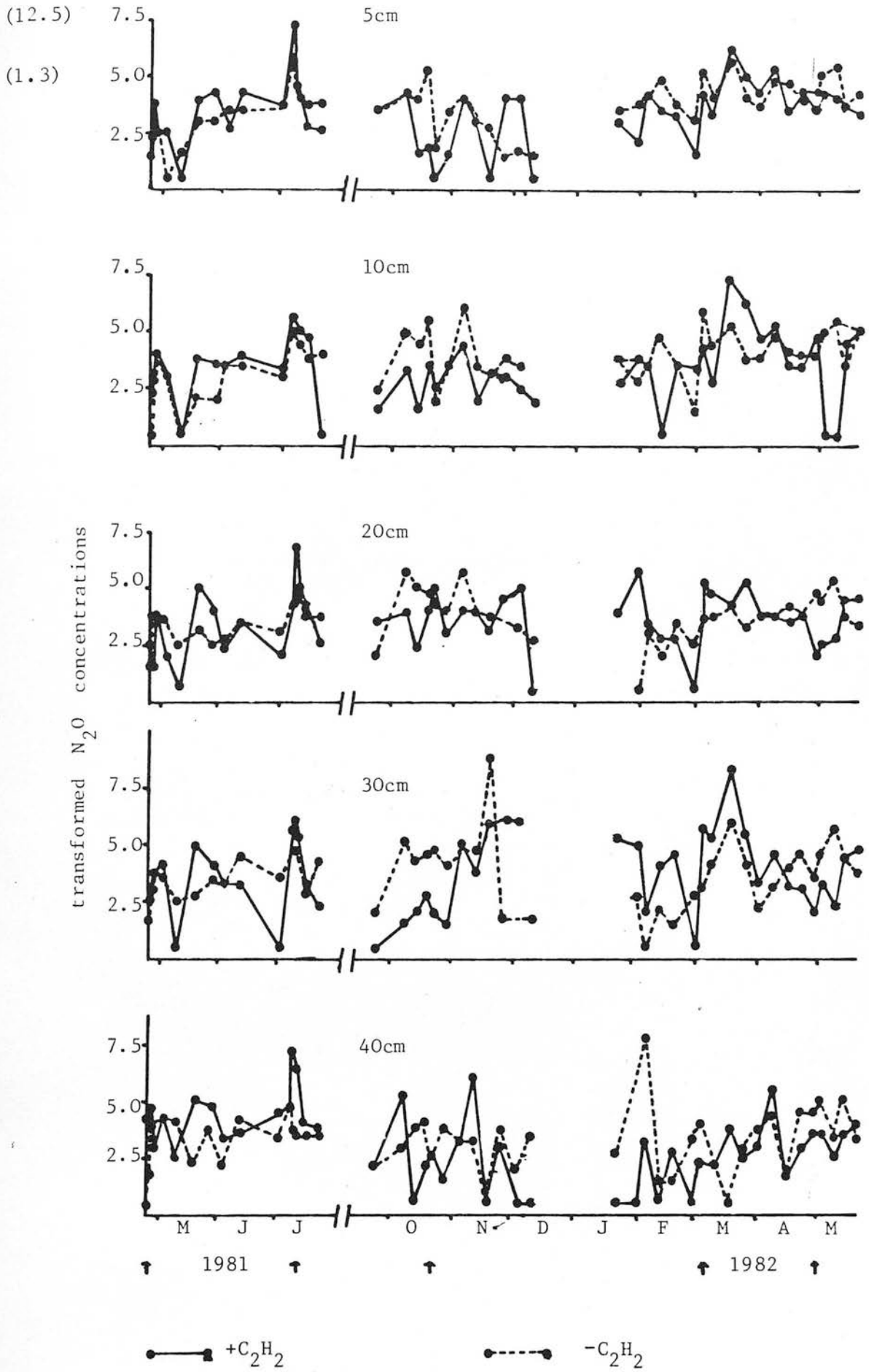


Fig. 8.22. Mean N_2O concentrations in slurried microplots with and without C_2H_2 (arrows indicate applications of slurry and fertiliser. Figures for N_2O given in brackets are the reverse transform of N_2O concentration (ml $ml^{-1} \times 10^6$)

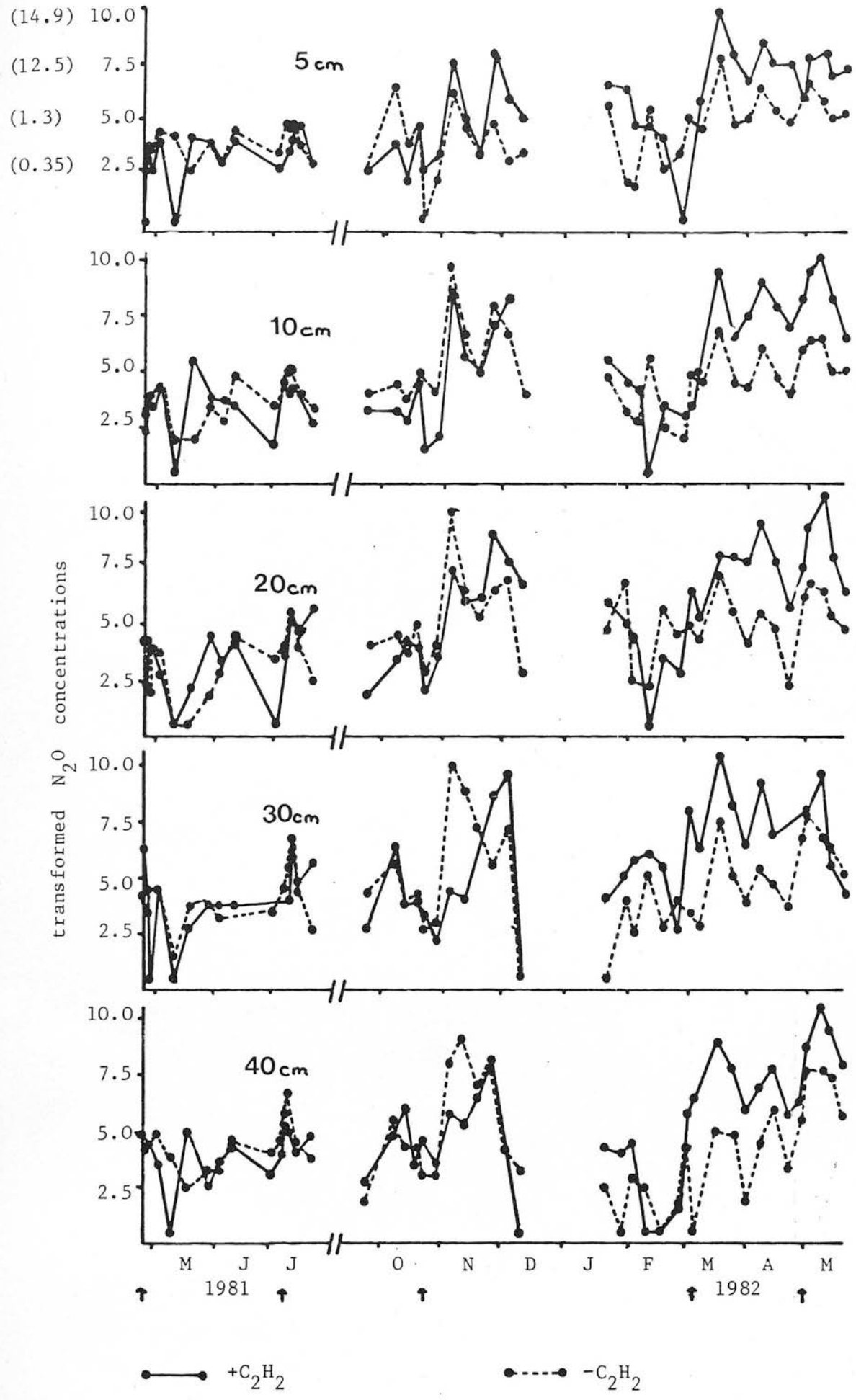


Fig. 8.23. Mean N_2O concentrations in fertilised microplots with and without C_2H_2. Arrows indicate applications of slurry and fertiliser. Figures for N_2O given in brackets are the reverse transform of N_2O concentration (ml ml^{-1} x 10^6)

large water applications in July 1981 and April 1982 had no effect on N_2O. There was no increase in N_2O concentrations following the thaw in January, in contrast to the data from the preliminary field experiment (Section 2.5), possibly because temperatures were so low at this time, even at the 30cm depth.

In the slurried plots also, N_2O concentrations were low throughout most of the experimental period and there was little response to applications except following those of July 1981 and March 1982, when N_2O concentrations increased for about a week. Concentrations of N_2O were highest in the spring, particularly at depths up to 20cm, but at other times concentrations were similar to or even below those of the controls. This is in contrast to previous field experiments where slurry increased N_2O concentrations (Sections 2.6 and 3.7).

In the fertilised plots, N_2O concentrations in the spring and summer of 1981 were very similar to those of the controls, except following the application of water and fertiliser in July 1981, when they increased for about 1 week at all depths. However, from late October to the beginning of December, and in March and April following fertiliser applications, N_2O concentrations were much higher than in the control or slurried treatments at all depths, and much higher where C_2H_2 was applied, with the highest concentrations being recorded at the 10, 20 and 30cm depths. Following the thaw N_2O concentrations were very low even at 30cm and 40cm, where O_2 was low at this time.

8.6. Flux Measurements

Since the frequency distribution of N_2O concentrations in the soil atmosphere was not normal, it is not surprising that N_2O fluxes were also not normally distributed (Figs. 8.24 and 8.25). Fluxes of up to 115g N ha^{-1} h^{-1} were measured. About 13% of the fluxes measured were apparently negative, i.e. less N_2O was trapped from the air coming from the cylinder headspace than from the atmosphere directly. This implied that the soil was acting as a sink for N_2O. However, since there was difficulty in setting identical flow rates for the flow of air through all the cylinders and control, it was concluded that this source of experimental error could account for the negative values.

Over 90% of the measured fluxes were below 5g N ha^{-1} h^{-1} (44kg N ha^{-1} a^{-1}). There was a marked difference in the median flux from

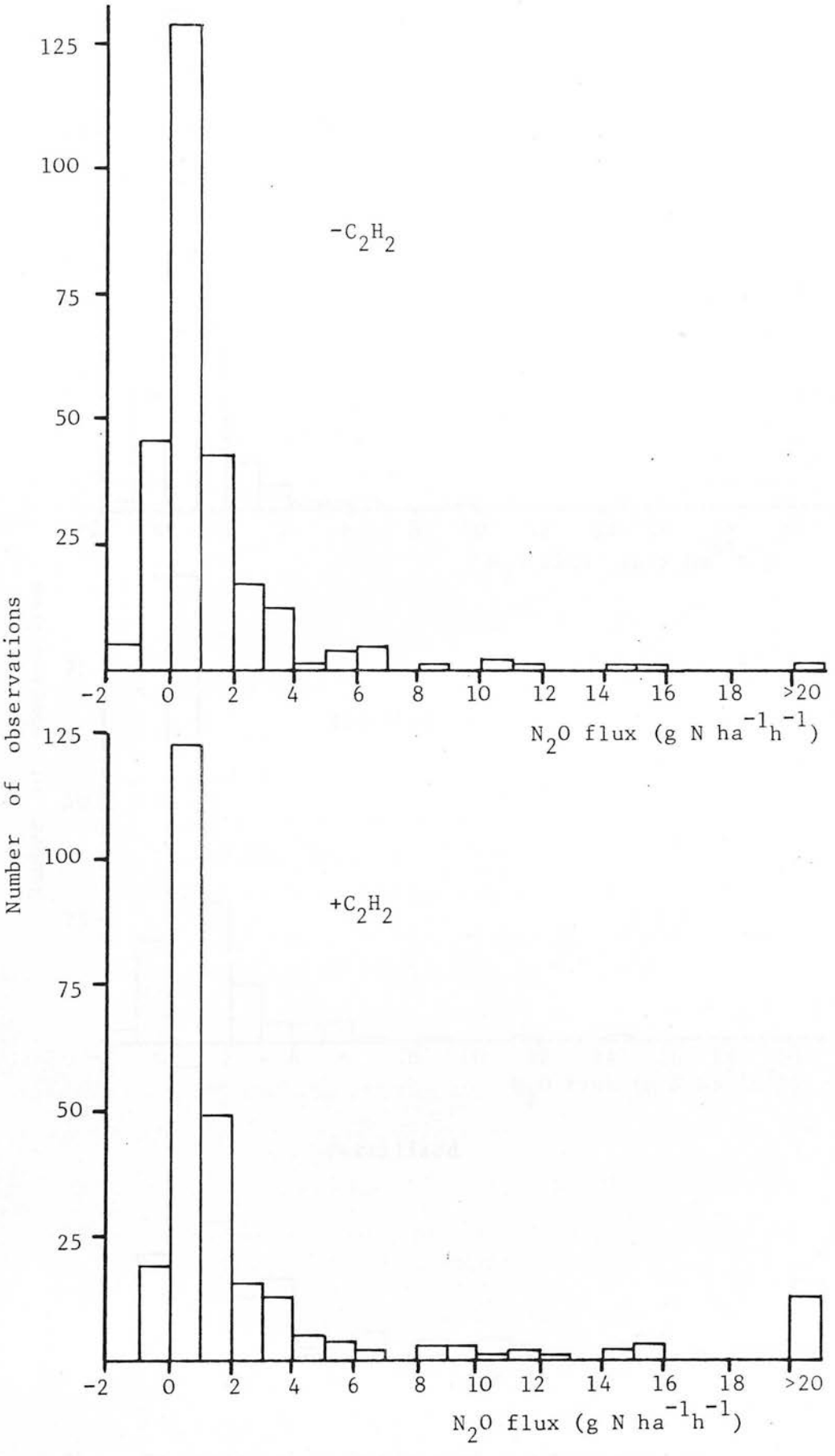


Fig. 8.24. Frequency distributions of N_2O flux data from micro-plots with and without C_2H_2

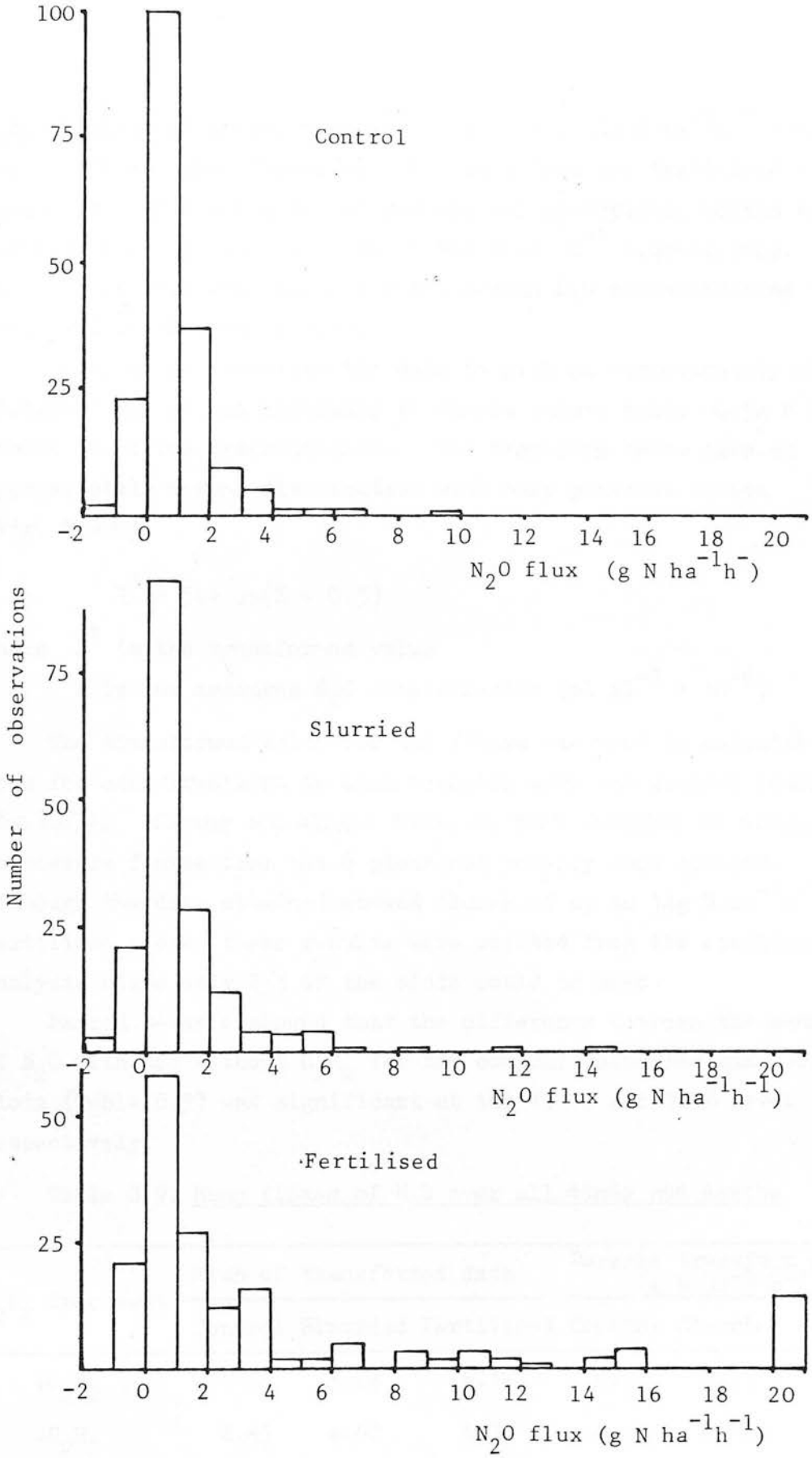


Fig. 8.25. Frequency distributions of N_2O flux data from control, slurried and fertilised microplots

C_2H_2 treated and untreated plots (0.85 and 0.64g N $ha^{-1}h^{-1}$ respectively.) Much higher fluxes were recorded from the fertilised microplots than from the slurried and control microplots, median values for fluxes being 1.15, 0.66 and 0.60g N $ha^{-1}h^{-1}$ respectively. This corresponds well with the order for median N_2O concentrations in the soil profile (Section 8.5.4).

In order to transform the data to give an approximately normal distribution, it was necessary to ignore values below -0.5g N $ha^{-1}h^{-1}$ (about 3% of the observations). The transform below gave an approximately normal distribution with only positive values (Fig. 8.2 6).

$$X^1 = 5 + 1n(X + 0.5) \qquad 8.3$$

where X^1 is the transformed value

X is the measured N_2O concentration (ml ml^{-1} x 10^{-6})

The transformed value for the fluxes was used to calculate the mean for each treatment on each occasion with and without added C_2H_2 (Fig.8.27). During the slight thaw, on 31st December an attempt was made to measure fluxes from the 8 plots not totally snow covered. Although the data obtained showed fluxes of up to 14g N $ha^{-1}h^{-1}$ in fertilised plots, these results were omitted from the statistical analysis since only 2/3 of the plots could be used.

Paired t-tests showed that the difference between the mean flux of N_2O with and without C_2H_2 for the control, slurried and fertilised plots (Table 8.9) was significant at the 1, 1, and 0.1% level respectively.

Table 8.9. Mean fluxes of N_2O over all times and depths

C_2H_2 treatment	Mean of transformed data			Reverse transform of mean (g N ha^{-1} h^{-1})		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised
$+C_2H_2$	5.08	5.24	6.17	0.59	0.77	2.73
$-C_2H_2$	4.69	4.62	5.35	0.30	0.18	0.92

The reverse transforms for the means on each occasion are plotted in Fig. 8.27.

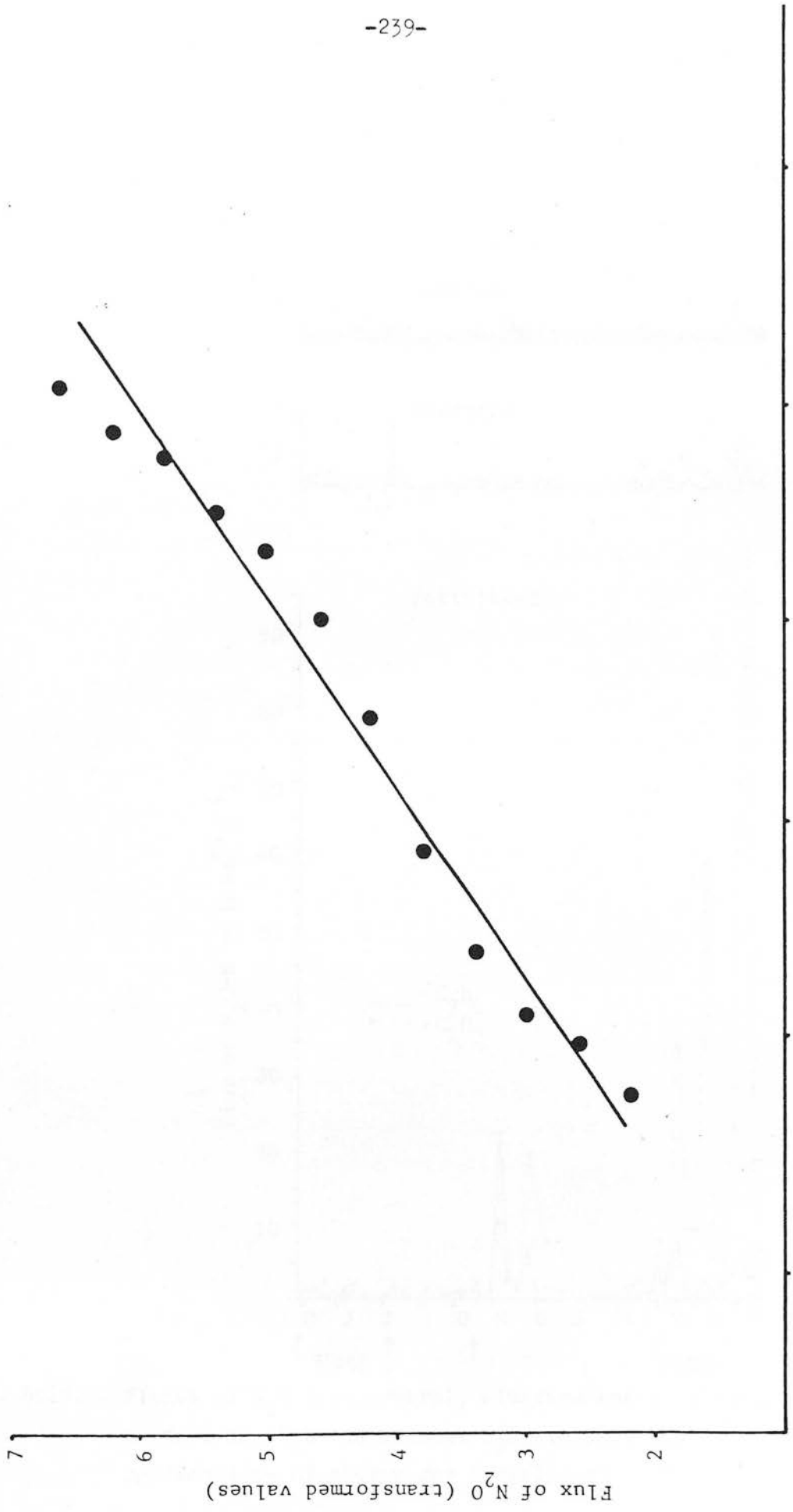


Fig. 8.26. Probability plot for transformed values of N_2O flux data

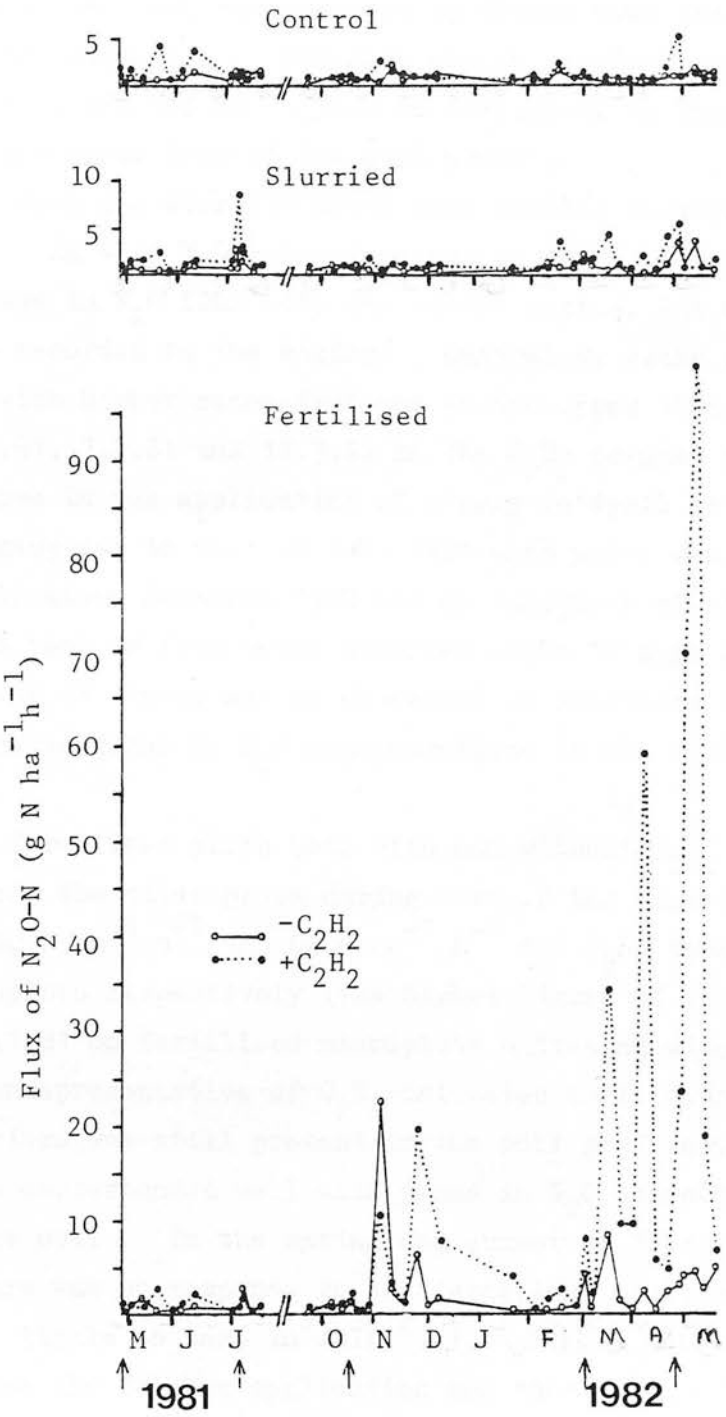


Fig. 8.27. Fluxes of N_2O from control, slurried and fertilised microplots with and without C_2H_2 (arrows indicate applications of slurry and fertiliser)

Fluxes were generally below 1g ha^{-1} h^{-1} (8.8kg N ha^{-1} y^{-1}) for most of the year from both C_2H_2- and non-C_2H_2-treated control plots (Fig. 8.27). As with N_2O concentrations in the soil profile, there was no response to the large volume of water applied to all lysimeters in July 1981 and May 1982, nor any increase in fluxes over the winter period, except at the beginning of November and in mid-February. The occasionally higher fluxes did not appear to correspond to higher N_2O concentrations in the upper 10cm of the soil profile.

Fluxes of N_2O from the slurried plots were similar to those of the control plots. As with N_2O concentrations in the soil profile, there was no increase in N_2O flux over the winter months, but slightly higher fluxes were recorded in the spring. Generally, peaks in the flux corresponded with higher concentrations in the upper 10cm of the soil (e.g. on 21.5.81, 7.7.81 and 17.3.82 in the C_2H_2 treated plots). There was no response to the application of slurry in April or October 1981 but a slight response to that of July 1981 when water was also applied. The application in March 1982 had no immediate effect but may have caused the peak in flux which occurred about 10 days later. The final application of slurry and water caused an immediate increase in flux although the increase in N_2O concentrations in the soil was small.

The flux from fertilised plots both with and without C_2H_2 was much higher than from the other plots during most of the experimental period, reaching 99g N ha^{-1} h^{-1} and 8g N ha^{-1} h^{-1} for C_2H_2 treated and untreated microplots respectively (the higher figure of 22.7g N ha^{-1} h^{-1} observed on 4.11.81 on fertilised microplots untreated with C_2H_2 was thought to be unrepresentative of C_2H_2-untreated soil, since C_2H_2 applied the week before was still present in the soil profile). The peaks in N_2O fluxes corresponded well with peaks in N_2O concentrations at 5 and 10cm in the soil. In the spring and summer of 1981, fluxes remained low. There was no response to the fertiliser application in April 1981 and very little to that in July 1981. Fluxes remained low until two weeks after the October application and then remained high until January. After the application in March 1982, fluxes remained very high until the end of the experimental period, and there was a marked increase where C_2H_2 was applied.

An analysis of variance for the mean flux from each plot over

Table 8.10. Analysis of Variance of Fluxes over 5 Periods

Dates	Source	df	ss	ms	F
28.4.81-12.5.81	C_2H_2 treatment	1	1.20	1.20	1.30
	Error	2	1.85	0.92	
	Total	3	3.05		
	N treatment	2	0.02	0.01	0.05
	N x C_2H_2 interaction	2	0.05	0.02	0.13
	Error	4	0.76	0.19	
	Total	11	3.88		
21.5.81-10.6.81	C_2H_2 treatment	1	1.19	1.19	8.81
	Error	2	0.27	0.13	
	Total	3	1.46		
	N treatment	2	0.55	0.27	2.63
	N x C_2H_2 interaction	2	0.30	0.15	0.71
	Error	4	0.84	0.21	
	Total	11	3.15		
2.7.82-21.7.81	C_2H_2 treatment	1	0.005	.005	2.00
	Error	2	0.005	.0025	
	Total	3	0.01		
	N treatment	2	0.39	0.19	5.85
	N x C_2H_2 interaction	2	0.30	0.15	4.61
	Error	4	0.13	0.03	
	Total	11	0.83		
23.9.81-28.10.81	C_2H_2 treatment	1	0.16	0.16	16.00
	Error	2	0.02	0.01	
	Total	3	0.18		
	N treatment	2	0.04	0.020	0.89
	N x C_2H_2 interaction	2	0.03	0.015	0.67
	Error	4	0.09	0.023	
	Total	11	0.34		
4.11.81-26.5.82	C_2H_2 treatment	1	2.00	2.00	2.74
	Error	2	1.46	0.73	
	Total	3	3.46		
	N treatment	2	6.77	3.39	10.51*
	(Fert. different	1	6.73	6.73	20.87*)[a]
	(from others				
	N x C_2H_2 interaction	2	0.32	0.16	0.50
	Error	4	1.29	0.32	
	Total	11	11.84		

a) Partitioning of sum of squares was by a method described by Pearce (1965)

the entire period was not meaningful since the C_2H_2 treatment was applied to different plots at different times. Therefore the mean flux for each plot was calculated for each period during which C_2H_2 was applied to the same 6 plots (Table 8.1). For each period an analysis of variance was carried out with C_2H_2 as the main treatment and the fertiliser regime as a subfactor (Table 8.10). This shows that the treatment differences were not significant except in the last period when the fertiliser significantly increased N_2O flux.

For most of the period when fluxes were low there was little difference between N_2O flux in the presence and absence of C_2H_2, as has been observed by other workers (Colbourn and Harper, 1982). The ratio of $N_2 + N_2O$ to N_2O flux, i.e. the flux with and without C_2H_2, was calculated for flux values of greater than 1g N ha^{-1} h^{-1}. The calculated ratio varied from 0.6 to 25, the higher values being associated with high total fluxes (Fig. 8.28). This indicates that when conditions are conducive to denitrification, i.e. the soil is very wet and NO_3^- concentrations are high, N_2 is the favoured product, probably because N_2O is retained longer by the soil and is therefore more likely to be reduced.

8.7. Estimation of Cumulative Flux

For each application of slurry and fertiliser, the subsequent cumulative N loss was calculated by assuming a constant flux between sampling occasions, and multiplying the N_2O flux by the time interval to the following measurement (Fig. 8.29). Between 9th December and 20th January when the soil at 10cm was continuously frozen, flux was assumed to be zero. This may have been an underestimate since during the slight thaw quite large N_2O fluxes were measured (Section 8.6).

The cumulative loss of N_2O in the presence of C_2H_2 was usually greater than in its absence. In the control and slurried plots, the estimated total N_2O flux for the entire period was 6.5 and 7.1kg ha^{-1} for C_2H_2-treated and 3.1 and 6.5kg ha^{-1} for untreated plots, respectively.

In the fertilised plots fluxes were high following the October 1981 application. The estimated proportion of applied N lost by denitrification (Table 8.11) was highest following the March 1982 application but the total loss was greatest following that of April, 1982.

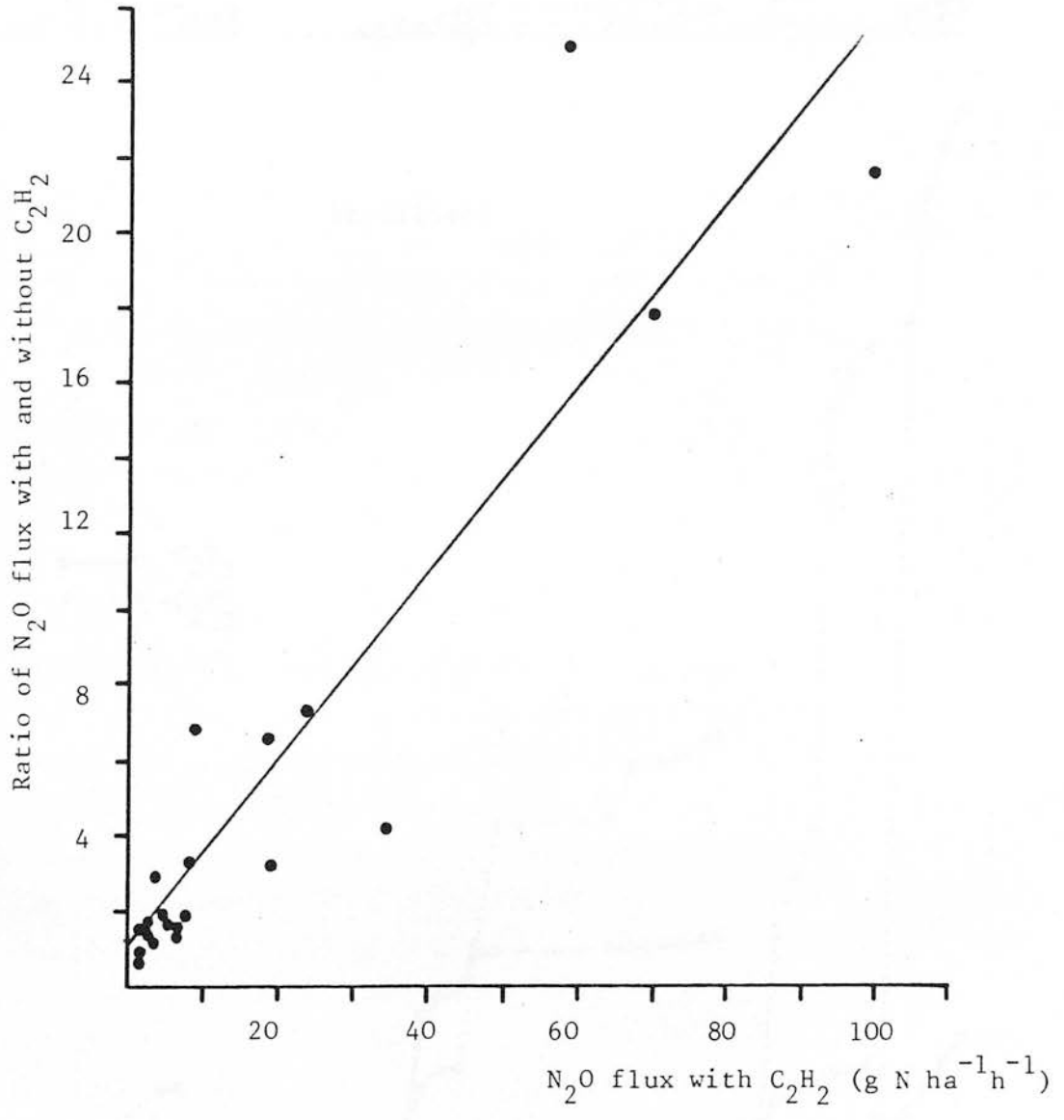


Fig. 8.28. Relationship between the ratio of N_2O flux with and without C_2H_2 to N_2O flux with C_2H_2 (showing regression line, $r2 = .843$)

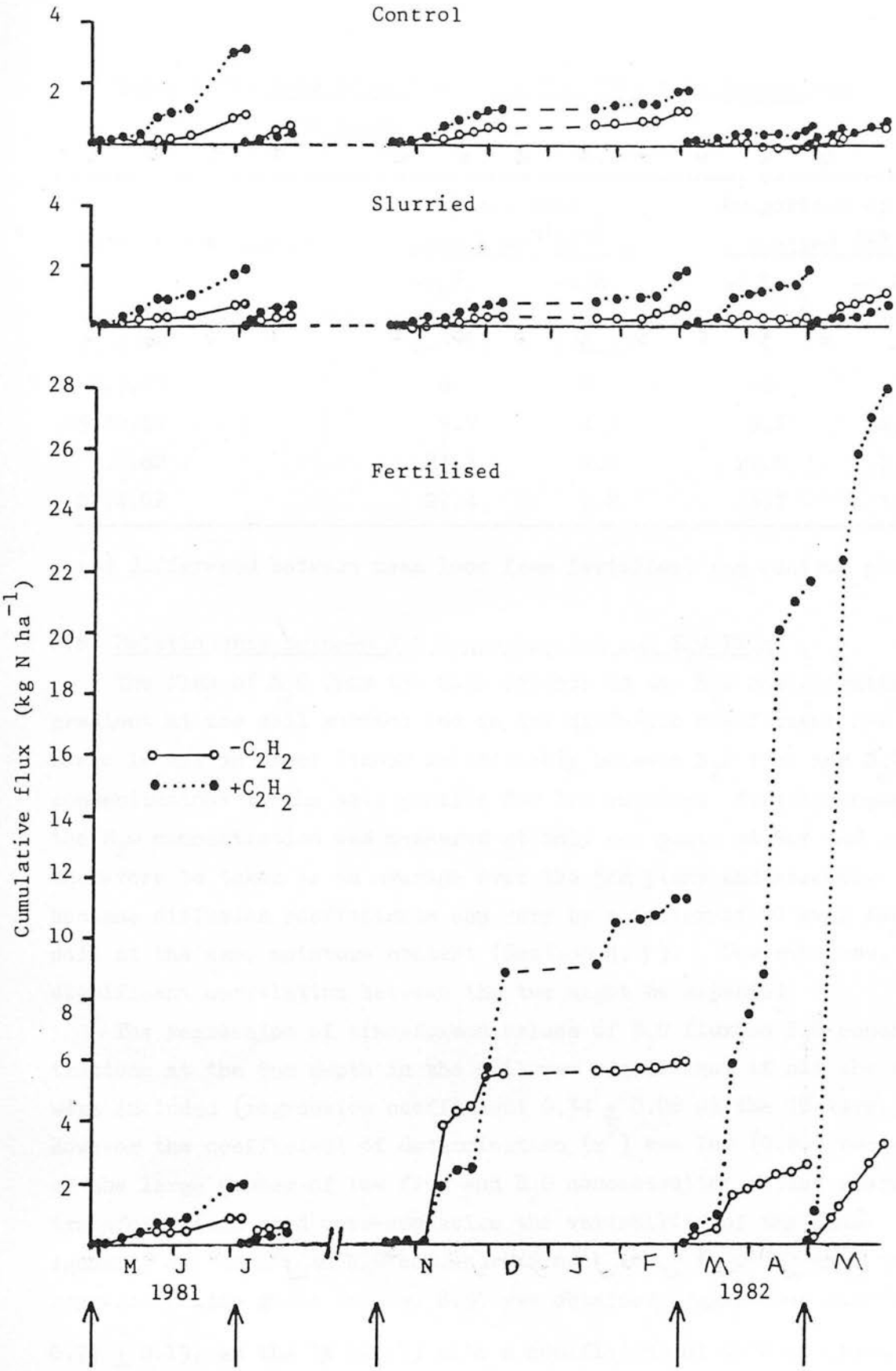


Fig. 8.29. Flux integrated over time following 5 fertiliser applications to microplots (arrows indicate applications of slurry and fertiliser)

Table 8.11. Loss of applied N by N_2O flux from fertilised microplots

Date of Application	Total loss (kg N ha^{-1})(a)		Proportion of N applied (%)	
	$+C_2H_2$	$-C_2H_2$	$+C_2H_2$	$-C_2H_2$
27.4.81	(-2.1)	(-0.2)	-	-
7.7.81	0	0	0	0
18.10.81	9.7	4.9	9.7	4.9
3.3.82	21.1	2.8	21.1	2.8
27.4.82	27.4	2.8	13.7	1.4

(a) difference between mean loss from fertilised and control plots

8.8. Relationship between N_2O Concentration and N_2O Flux

The flux of N_2O from the soil depends on the N_2O concentration gradient at the soil surface and on the diffusion coefficient for N_2O. There is not an exact linear relationship between N_2O flux and N_2O concentrations in the soil profile for two reasons: firstly because the N_2O concentration was measured at only one point at 5cm and cannot therefore be taken as an average over the 5cm plane and secondly because diffusion coefficients can vary by a factor of 10 even for soil at the same moisture content (Section 4.3). Nevertheless, a significant correlation between the two might be expected.

The regression of transformed values of N_2O flux on N_2O concentrations at the 5cm depth in the soil was significant if all the data were included (regression coefficient 0.34 ± 0.08 at the 1% level). However the coefficient of determination (r^2) was low (0.20) because of the large number of low flux and N_2O concentration values where the transformations used over-emphasise the variability of the data. By ignoring all data with N_2O concentrations below 1×10^{-6} ml ml^{-1} the regression line given in Fig. 8.30 was obtained (regression coefficient 0.77 ± 0.19, at the 1% level) with a coefficient of determination (r^2) of 0.49.

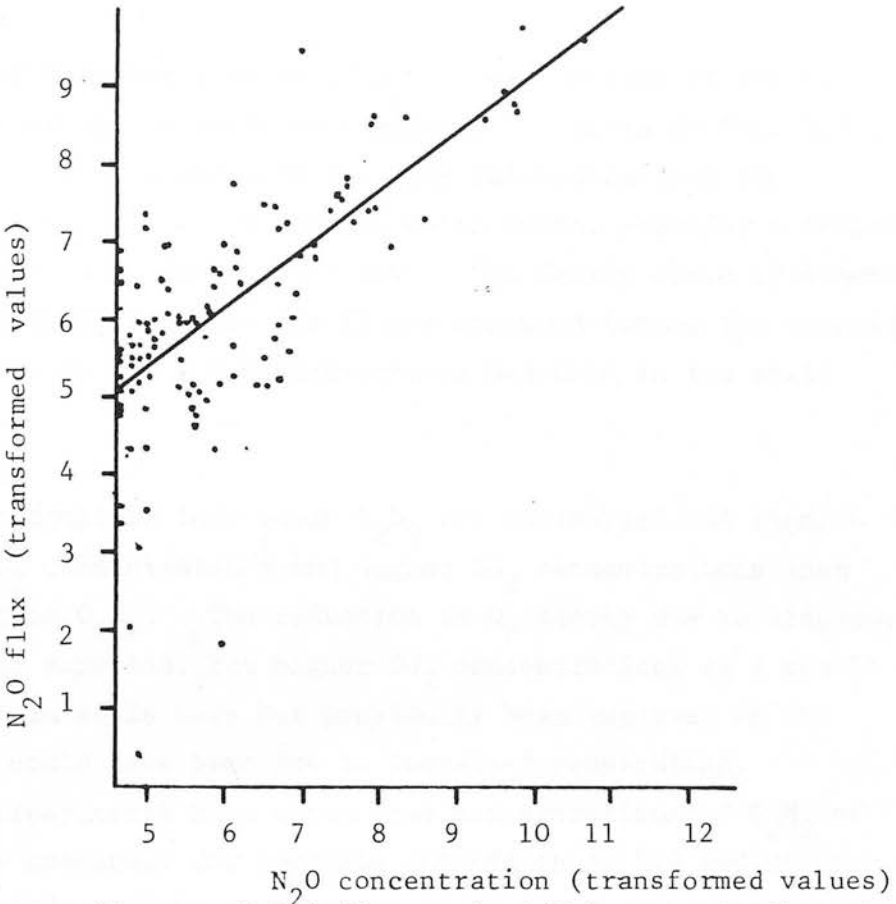


Fig. 8.30. Scatter diagram of N_2O flux against N_2O concentration at the 5cm depth showing regression line.

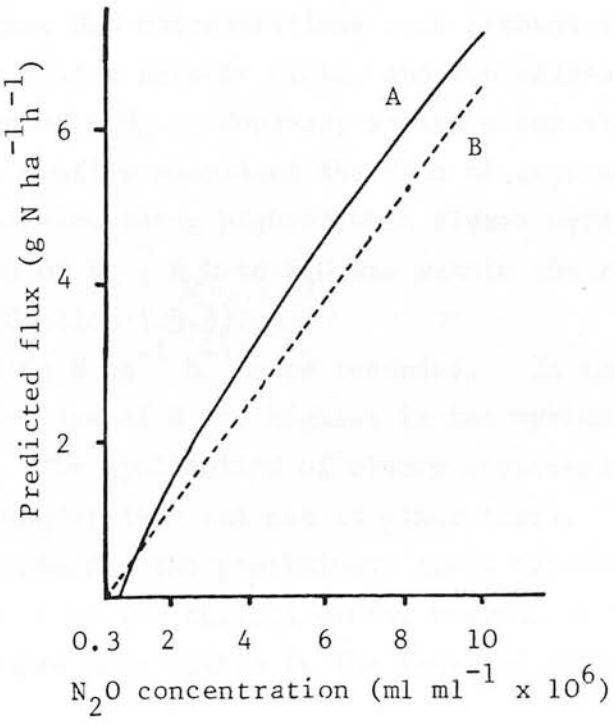


Fig. 8.31. Relationship between N_2O flux and N_2O concentration at the 5cm depth in the soil profile

A. given by the regression line (Fig. 8.30)

B. calculated from Fick's 1st Law assuming a moisture tension of 5k Pa

The flux of N_2O over a range of N_2O concentrations at the 5cm depth as predicted by the regression equation is given in Fig. 8.30, and in Fig. 8.31 is compared with the flux calculated from the measured diffusion coefficient for the 0-5cm depth, assuming a tension of 5k Pa (Section 4.3) using Fick's Law. The fairly close agreement between the two indicates that the fluxes measured during the experiment were compatible with the N_2O concentrations measured in the soil.

8.9. Conclusions

Enclosed microplots into which C_2H_2 was introduced had significantly lower O_2 concentrations and higher CO_2 concentrations than those receiving no C_2H_2. The reduction in O_2 simply due to displacement by C_2H_2 was expected, but higher CO_2 concentrations as a result of C_2H_2 used in field soils have not previously been reported in the literature and could have been due to increased respiration.

Previous experiments have shown that concentrations of C_2H_2 of 0.05ml ml^{-1} are necessary for complete inhibition of N_2O reduction (Section 6.4). Since C_2H_2 concentrations at some depths were lower than this, fluxes of gaseous N_2 may have been underestimated, particularly if the major source of N_2O was at greater depths in the soil. This is probable since N_2O concentrations were highest at 20 and 30cm.

High random variation between plots, and low fluxes, frequently obscured the effects of C_2H_2. However, it was clear that the presence of C_2H_2 in the soil profile increased the flux of gaseous N by a factor of up to 25, the increase being highest when fluxes were highest. The range of flux ratios of $N_2 + N_2O$ to N_2O was within the range reported in the literature (Section 1.5.3).

Fluxes up to 115g N ha^{-1} h^{-1} were recorded. In the control plots the total integrated loss of N was highest in the spring of 1981 and winter of 1981/82. The application of slurry increased the N loss slightly in the spring of 1982 but not at other times. Fluxes estimated from the data for the preliminary field experiment were similar from the slurried and control plots, whereas in the randomised block experiment fluxes were higher in the slurried plots than in the

control plots. The application of inorganic fertiliser increased the flux of N from the beginning of winter until the end of the experimental period, with up to 20% of the applied N being lost as N_2O in C_2H_2-treated plots. The range of observed fluxes and the loss of N was within the range of values reported in the literature (Table 1.5).

There was no evidence of a decreasing difference in flux between C_2H_2 treated and untreated plots over the experimental period, indicating that the soil microflora did not adapt to the presence of C_2H_2 as has been suggested in the literature (Section 1.2.4.1).

In the absence of C_2H_2 the total integrated fluxes of N_2O over the experimental period were intermediate between the predicted fluxes for the '79/'80 and '80/'81 data for the control and slurried treatments and similar to that for the '80/'81 data for the fertilised microplots (Section 4.5).

The regression of N_2O fluxes against N_2O concentrations at 5cm in the soil profile was significant. It was shown that the fluxes measured could reasonably be expected to result from the N_2O concentrations measured at the 5cm depth. Such a comparison between flux measurements and values calculated from soil atmospheric analysis has not been published previously.

Published estimates of N_2O flux have shown that they are highest in the autumn and spring and low in winter and summer (unless irrigation is used (Section 1.5.3). This was confirmed by the results reported here but contrasts with the fluxes predicted from the data for the previous two years where N_2O loss was highest during the winter. This may have been because in the calculation of fluxes the diffusion coefficient was assumed to be constant over the entire period, whereas in fact during the winter the soil moisture tension, and therefore the diffusion coefficient may have been lower than in the autumn and spring.

The "background" fluxes, i.e. the low N_2O fluxes recorded for most of the period were below 2g N ha^{-1} h^{-1} and agree well with the results of Ryden (1983), Webster and Dowdell (1982), and Armstrong (1982) who obtained values of 8.3 and 2.1 and 0.04g N ha^{-1} h^{-1}, respectively.

9. SUMMARY AND GENERAL CONCLUSIONS

The composition of the soil atmosphere was investigated over three periods: September 1978 - June 1979, July 1979 - May 1980, and April 1981 - May 1982. Although the three experiments were designed differently, the results can be compared qualitatively, and show how aeration and the occurrence of N_2O vary from year to year and how inorganic fertiliser and slurry affect concentrations of O_2 and N_2O in the soil.

Generally, concentrations of O_2 decreased with depth in the soil, the difference between those at 15 and 30cm being greater than between 30 and 45cm. In all years O_2 concentrations were high during the summer at all depths and decreased over the winter. The severity of the decrease, and the length of time during which O_2 concentrations were depressed, were strongly influenced by the pattern of rainfall. Thus the first occurrence of low O_2 concentrations was much earlier in the wet autumn of 1978 than in the drier years of 1979 and 1981. Similarly, the very wet spring of 1979 prolonged the period of low O_2, and, coupled with the increase in biological activity in the spring, resulted in the lowest O_2 concentrations occurring in May. In contrast, in the other years, near ambient concentrations had been re-established at this time.

During the first experiment, one large slurry application in September decreased O_2 concentrations at all depths for several weeks. In subsequent years smaller applications were made more frequently. Decreases in O_2 were measureable (but small) following applications in the autumn or spring, but not in the summer.

There was a general inverse relationship between O_2 and N_2O concentrations in the soil. Regression analysis, using data from the first two experiments, showed that the relationship was significant at all depths for all treatments. At the 15cm depth, N_2O concentrations were usually low, corresponding with the higher O_2 concentrations. However, although O_2 concentrations were generally lowest at the 45cm depth, N_2O concentrations were similar at the

30 and 45cm depth. Although N_2O concentrations were higher at depth in the soil, this does not necessarily imply increased denitrification. Measured diffusion coefficients for the 40cm depth were an order of magnitude lower than in the surface soil at the same moisture tension, resulting in a build up of N_2O. Incubation experiments showed that rates of NO_3^- reduction were much lower in soil from greater depths, although the potential for N_2O reduction did not decrease as much.

In the untreated plots, N_2O concentrations were always low in the summer, and increased over the winter, the increase corresponding to the decrease in O_2 concentrations. The highest concentrations were observed in the first experiment, where N_2O concentrations reached a peak following a period when the surface soil was frozen. This showed that denitrification could take place at very low temperatures. In subsequent years there was no such peak, even though, during the third experiment, there was again a long period when the surface soil was frozen, and N_2O concentrations were lower during the winter (corresponding to higher O_2 concentrations) than during the first year.

Slurry applications made during the summer did not cause increased N_2O concentrations, in contrast with applications in the autumn and spring, when the increase in N_2O depended on soil moisture conditions and corresponded to the decrease in O_2. The greatest response followed a large slurry application in the wet autumn of 1978.

There was never any evidence from soil analysis of greatly increased NH_4^+ or NO_3^- concentrations in the soil profile as a result of applications of slurry, even where cores were taken a few weeks following the applications. The low NH_4^+ concentrations indicate rapid nitrification of the NH_4^+ initially present in the slurry in contrast to the results of Thijell and Burford (1975) (Section 1.5.1.4). The immediate effects of slurry on denitrification were therefore due to NO_3^- released from the NH_4^+ in the slurry as well as to lower O_2 concentrations caused by water in the slurry. Since high N_2O concentrations were found at all depths

in the soil, whereas slurry N would be expected to remain on the surface, the water was probably the major influence on N_2O concentrations. The low NO_3^- concentrations indicate rapid loss by crop uptake, leaching and denitrification. The slow release of N from the slurry organic matter may have led to the long term increases in N_2O concentrations in slurried plots over the winter. Since only half of the N in slurry is in the organic form and approximately half of this is mineralised in the first year, and mineralisation is slow over the winter (Section 1.5.1.4) such increases in N_2O probably represented a small N loss.

As with slurry, the application of inorganic fertiliser during the summer did not influence N_2O, but in the autumn and spring often increased N_2O concentrations, the increase depending on the soil moisture conditions. The highest N_2O concentrations were found following an application in the wet autumn of 1978, and in the spring of 1982. Increases were greatest at the 30 and 45cm depths, indicating that some of the NO_3^- leached rapidly down to these depths following applications in wet conditions. During the winter N_2O concentrations were highest in fertilised plots, indicating that some NO_3^- remained in the profile at the end of the growing season.

The immediate increase in N_2O as a result of slurry applications was greater than or similar to that for inorganic fertiliser during the first two years but much less in the third experiment, while the long term effects of the slurry, e.g. over the winter, were always much less than for inorganic fertiliser.

Although fluxes of N_2O were not measured directly in the first two experiments, estimated fluxes were calculated from Fick's 1st Law using measured diffusion coefficients and N_2O concentrations at the 15cm depth. In the first year calculated fluxes were 2.7, 3.1, and 6.0kg N ha^{-1} for the control, slurried and fertilised plots respectively, assuming a soil moisture tension of 5kPa (except on one occasion following the thaw in 1979 when soil moisture tension was assumed to be 1kPa), or 1.3, 1.3, and

2.7kg N ha^{-1} assuming a soil moisture tension of 1kPa. Most of the flux occurred immediately following the fertiliser applications and following the thaw in February 1979. Thus in spite of the large slurry application, N_2O-N losses were much less for the slurried than for the fertilised plot. In the second year, where more frequent applications of slurry and fertiliser were made, calculated fluxes were much lower: 0.8, 1.9 and 2.0kg N ha^{-1} for the control, slurried and fertilised plots respectively at 5kPa tension, and 0.2, 0.4, and 0.5kg N ha^{-1} at 1kPa tension. Most of the loss occurred in the autumn from the slurried plot, and over the winter from the fertilised plots. Although no measurements of soil moisture tension were carried out during the first two experiments, measured soil moisture tensions in the third winter period (Fig. 8.7) would indicate that at the 10 and 20cm depth tensions were closer to 1kPa than to 5kPa during most of the winter period. Actual fluxes were therefore closer to the lower than the upper limits given above.

In the first two field experiments no measurements were made of the ratio of total N flux from denitrification to N_2O-N flux. However if an average ratio of 10 is assumed (Section 1.5.3), the losses quoted above represented a substantial proportion of the fertiliser-N applied.

In the third experiment, fluxes of N_2O were measured directly by trapping N_2O diffusing into the headspace of enclosed microplots. By using C_2H_2, both N_2O and total N flux could be measured. The method adopted for introducing C_2H_2 into the soil ensured that C_2H_2 reached to at least the 40cm depth. Fluxes over most of the experimental period were low, corresponding to the low N_2O concentrations in the soil profile during the experiment and the relatively good aeration compared to other years. Total losses of N_2O-N from the control and slurried plots over the experimental period were estimated to be 6.5 and 7.1kg ha^{-1} respectively when C_2H_2 was present - only slightly higher than in its absence. Losses of N were much higher from the fertilised microplots,

especially in the autumn and spring, and there was a much greater difference between C_2H_2 treated and untreated plots, 56.1kg N ha^{-1} being lost from C_2H_2 treated plots compared with 10.3kg N ha^{-1} from untreated plots over the experimental period. Fluxes of up to 115g N $ha^{-1}h^{-1}$ were recorded and about 21% of the N applied on one occasion was lost by denitrification. It was shown that the measured fluxes could reasonably have arisen from the measured N_2O concentrations in the soil profile. The ratio of total flux to loss of N_2O alone increased with total flux, values of up to 25 being recorded, indicating that under conditions conducive to denitrification, N_2O was more likely to be reduced to N_2, while where fluxes were low most loss was as N_2O.

The flux measurements therefore confirm the results of soil atmospheric analysis, i.e. that losses of N by denitrification are most likely to result from applications of fertiliser in the autumn and spring and that the highest fluxes result from NO_3^- fertiliser. These observations are in broad agreement with those from other comparable studies for rain fed agricultural systems.

A comparison of N_2O-N losses following applications of inorganic fertiliser and slurry undertaken by Sandford (1980) showed that losses of N were 7.5 times greater with inorganic fertiliser than with slurry. In the present study N_2O losses were only 1.6 times greater in the absence of C_2H_2 but 7.9 times greater when C_2H_2 was used and overall losses were much higher.

Few studies to measure total denitrification as well as N_2O loss have been carried out in Britain. Ryden (1983) reported fluxes of N_2O using an C_2H_2 technique, on loamy grassland soil, with annual applications of 0, 250 and 500kg N ha^{-1} as NH_4NO_3 in equal amounts during the year. Ryden's results were broadly similar to those reported here. He found a range of fluxes in C_2H_2-treated sites up to 83g N $ha^{-1}h^{-1}$ (cf. 117g N $ha^{-1}h^{-1}$ in the study reported here) and "background" rates, i.e. when NO_3^- or soil moisture conditions limited denitrification, of <2g N $ha^{-1}h^{-1}$ (cf. <1g N $ha^{-1}h^{-1}$). The greatest losses of applied fertiliser N in his study occurred in July 1980 (in a very

wet summer) and March 1981 when 8.4% and 15.6% respectively was lost, corresponding to times when soil moisture contents were high and rainfall occurred following fertiliser applications. These losses were similar in magnitude to those found in this study: 10% in autumn, 1981 and 21% in spring, 1982. Ryden found evidence of negative fluxes occurring consistently for long periods in both the unfertilised plots and those receiving 250kg N $ha^{-1}a^{-1}$, where NO_3^- concentrations were low, temperatures above 5^oC and the moisture content was above 20%. In the present study, negative fluxes were measured but attributed to experimental error.

In contrast to the results reported here, Ryden (1983) always found higher fluxes under the C_2H_2 treatment, the proportion of N lost as N_2O being highest when total flux was highest: 15-79% of total loss was as N_2O. He attributed this to N_2O being favoured as the product of denitrification when NO_3^- concentrations were high. Although he did not measure N_2O concentrations in the soil profile, he produced evidence to show that most denitrification occurred in the upper 20cm of the soil profile. In this case the shorter diffusion pathway may have led to higher N_2O fluxes. It is also possible that his methods of introducing C_2H_2 into the soil did not establish adequate C_2H_2 concentrations at lower depths in the soil, i.e. that total flux was underestimated. In contrast with Ryden's observations, in the present study it was found that gaseous loss of N was principally as N_2 when the rates of denitrification were high. There was evidence that high N_2O concentrations at depth in the soil, which is likely to be reduced to N_2 during diffusion to the surface, contributed considerably to total N flux. Colbourn and Harper (1982) who used an C_2H_2 technique similar to that of Ryden to measure fluxes in autumn and over one winter, also found higher ratios of total:N_2O flux (7-10) when total flux was high, while at low fluxes there was little difference between fluxes from C_2H_2 treated and untreated sites.

Other workers have measured N_2O fluxes only: e.g. Armstrong (1983), using two soils irrigated to field capacity, applied 200kg ha^{-1} as calcium nitrate in October and measured subsequent fluxes over the winter, while Webster and Dowdell (1982), using lysimeter monoliths with two soils, measured fluxes over a three year period with applications of 0, or 400kg N $ha^{-1}a^{-1}$ as inorganic fertiliser. Measured maximum N_2O fluxes in Armstrong's work were 6.7 and 0.2g N $ha^{-1}h^{-1}$ in a clay and loamy sand respectively, much lower than the fluxes of up to 10g N $ha^{-1}h^{-1}$ found by Webster and Dowdell and up to 8g N $ha^{-1}h^{-1}$ reported here. Both Webster and Dowdell and Armstrong found rates were highest after fertilised application to wet soil and low during the winter. "Background rates" of N_2O emission reported by Armstrong (0.04g N $ha^{-1}h^{-1}$) and by Webster and Dowdell (0.1-0.2g N $ha^{-1}h^{-1}$) were more precise than those reported here because their measurement technique was more sensitive. In contrast to Ryden's work they did not report negative fluxes.

The paucity of quantitative data of denitrification losses, particularly following applications of slurry and other organic fertilisers, illustrates the fact that denitrification is the least understood process in the nitrogen cycle. More work is needed in particular to improve the C_2H_2 technique and adapt its use to heavy clay soils and to quantify and predict N losses under different soils and management practices.

The effects of C_2H_2 on nitrification and respiration were investigated. In a two week incubation of aerobic soil with C_2H_2 at a concentration of 0.04ml ml^{-1} C_2H_2 totally inhibited nitrification, causing a build up of NH_4^+, and reduced mineralisation. In a longer term incubation, the presence of C_2H_2 resulted in a decrease of inorganic N to almost zero, but the effect was reversible. Previous published work has shown the reversible inhibition of nitrification but not the large decrease in inorganic N (Section 1.2.4.4). When C_2H_2 was in contact with soil for 1 day per week for several weeks, nitrification was reduced at a concentration of 0.05ml ml^{-1} but not at 0.005ml ml^{-1}. Thus, at higher concentrations, a recovery time of 6 days may have been

insufficient and in the field experiment using C_2H_2, where C_2H_2 was also in contact with the soil for 1 day per week, it is possible that nitrification may have been reduced. Since denitrification depends on NO_3^- concentrations, this may have led to an underestimate of N_2O flux in C_2H_2-treated plots, particularly in the control and slurried treatments. When the C_2H_2 treatment was switched from one set of microplots to another, recovery of nitrification would be expected within two weeks.

In laboratory incubations of anaerobic soil exposed to C_2H_2 for 1 day per week, there was no evidence of a lessening of the inhibitory effect of C_2H_2 on N_2O reduction, even after 6 weeks. Similarly, in the field experiment, since the ratio of N_2O flux with and without C_2H_2 did not decrease over the experimental period, C_2H_2 appeared to be effective even after several months.

In aerobic conditions C_2H_2 increased respiration in soil incubations and caused the depletion of inorganic N. Experiments confirmed that this was associated with the consumption of C_2H_2, probably by soil bacteria. Bacterial cultures were isolated from the soil, which could grow by using C_2H_2 as the sole carbon source. More than one bacterial species was found on the agar culture plates. Incubations in liquid culture confirmed beyond reasonable doubt that the bacteria consumed C_2H_2. Since this work was done, similar results have been reported by other workers (Colbourn et al., 1982). During the field experiment using C_2H_2, microplots to which C_2H_2 was applied had higher concentrations of CO_2 in the soil profile, particularly towards the end of the experimental period. It was possible, therefore, that the population of bacteria able to use C_2H_2 as a carbon source increased in the soil during the course of the experiment.

BIBLIOGRAPHY

Allan, M.B. 1952 Experiments on bacterial denitrification. J. Bacteriol. 64 397-412.

Allison, F.E. 1963 Losses of gaseous nitrogen from soils by chemical mechanisms involving nitrous acid and nitrites. Soil Sci. 96 404-409.

Allison, F.E. 1966 The fate of nitrogen applied to soils. Adv. Agron. 18 219-258.

Ardakani, M.S., Belser, L.W. and McLaren, A.D. 1975 Reduction of nitrate in a soil column during continuous flow. Soil Sci. Soc. Amer. Proc. 39 290-294.

Armstrong, A.S.B. 1983 Nitrous oxide emissions from 2 sites in Southern England during winter 1981/82. J. Sci. Food Agric. 34 803-807.

Armstrong, W. 1975 Waterlogged soil. In Environmental and Plant Ecology. Ed. Etherington, J.R. Wiley and Sons, London. 181-218.

Arnold, P.W. 1954 Losses of nitrous oxide from soil. J. Soil Sci. 5 116-128.

Asano, A. 1959 Studies on enzymic nitrite reduction. I. Properties of the enzyme system involved in the process of nitrite reduction. J. Biochem. (Tokyo) 46 781-790.

Avery, B.W. 1980 Soil classification for England and Wales (higher categories). Soil Survey Tech. Mono. 14.

Baalsrud, K. and Baalsrud, K.S. 1954 Studies on Thiobacillus denitrificans. Arch. Mikrobiol. 20 34-62.

Bailey, L.D. and Beauchamp, E.G. 1973 Effects of temperature on nitrate and nitrite reduction, nitrogenous gas production and redox potential in a saturated soil. Can. J. Soil Sci. 53 213-218.

Bailey, L.D. 1976 Effects of temperature and roots on denitrification in a soil. Can. J. Soil Sci. 56 79-87.

Balasubramanian, V. and Kanehiro, Y. 1976 Denitrification potential and pattern of gaseous nitrogen loss in tropical Hawaiian soils. Trop. Agric. (Trinidad) 53 293-303.

Baldensperger, J. and Garcia, J.L. 1975 Reduction of oxidized inorganic nitrogen compounds by a new strain of Thiobacillus denitrificans. Arch. Microbiol. 103 31-36.

Balderston, W.L., Sherr, B. and Payne, W.J. 1976 Blockage by acetylene of nitrous oxide reduction by Pseudomonas perfectomarinus. Appl. Environ. Microbiol. 31 504-508.

Ball, B.C. 1979 Characterisation of soil pores by gas flow and diffusion. Ph.D. Thesis Reading Univ. 1979.

Ball, B.C., the late Harris, W. and Burford, J.R. 1981 A laboratory method to measure gas diffusion and flow in soil and other porous materials. J. Soil Sci. 32 323-333.

Barbaree, J.M. and Payne, W.J. 1967 Products of denitrification by a marine bacterium as revealed by gas chromatography. Mar. Biol. 1 136-139.

Barsdate, R.J. and Alexander, V. 1975 The nitrogen balance of arctic tundra. Pathways, rates and environmental implications. J. Environ. Qual. 4 111-117.

Beijerinck, M.W. 1904 Phenomenes de reduction produits par les microbes. Arch. Neerl. Sci. Exactes Nat. 9 131-157.

Beijerinck, M.W. and von Minkman, D.C.J. 1910 Bildung und Verbrauch von Stickoxydul durch Bakterien. Zentralbl. Bakteriol. II Abt. 25 30-65.

Belford, R.K. 1979 Collection and evaluation of large soil monoliths for soil and crop studies. J. Soil Sci. 30 363-373.

Bell, R.G. 1969 Studies on the decomposition of organic matter in a flooded soil. Soil Biol. Biochem. 1 105-116.

Betrand, A.R. and Kohnke, H. 1957 Subsoil conditions and their effect on oxygen supply and the growth of corn roots. Soil Sci. Soc. Amer. Proc. 21 135-140.

Birch, H.F. 1958 The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10 9-31.

Birch, H.F. 1959 Further observations on humus decomposition and nitrification. Plant Soil 11 262-286.

Birch-Hirschfeld, L. 1932 Die umsetzung von acetylen durch Mycobacterium laticola. Zentralbl. Bacteriol. Parasitenkd. Infekionskr. Hyg. Abt. 285 113-130.

Blackmer, A.M. and Bremner, J.M. 1976 Potential of soil as a sink for atmospheric nitrous oxide. Geophys. Res. Lett. 3 739-742.

Blackmer, A.M. and Bremner, J.M. 1978 Inhibitory effect of nitrate on reduction of nitrous oxide to nitrogen by soil microorganisms. Soil Biol. Biochem. 10 187-191.

Blake, G.R. and Page, J.B. 1948 Direct measurement of gaseous diffusion in soil. Soil Sci. Soc. Amer. Proc. 13 37-41.

Bollag, J.M., Orcutt, M.L. and Bollag, B. 1970 Denitrification by isolated soil bacteria under various environmental conditions. Soil Sci. Soc. Amer. Proc. 34 875-879.

Bollag, J.M. and Tung, G. 1972 Nitrous oxide release by soil fungi. Soil Biol. Biochem. 4 271-276.

Bollag, J.M., Drzymala, S. and Kardos, L.T. 1972 Biological versus chemical nitrite decomposition in soil. Soil Sci. 116 44-50.

Bowman, R.A. and Focht, D.D. 1974 The influence of glucose and nitrate concentrations upon denitrification rates in a sandy soil. Soil Biol. Biochem. 6 297-301.

Boyce, J.S. and McCalla, T.M. 1969 Aeration status of subtilled and ploughed soils as determined by the potential vacuum method. Soil Sci. 108 241-248.

Boynton, D. and Reuther, W. 1938 A way of sampling soil gases in dense subsoils and some of its advantages and limitations. Soil Sci. Soc. Amer. Proc. 3 37-42.

Bremner, J.M. and Shaw, K. 1958 Denitrification in soil: I. Methods of investigation. J. Agric. Sci. 51 22-39.

Bremner, J.M. and Shaw, K. 1958 Denitrification in soil: II. Factors affecting denitrification. J. Agric. Sci. 51 40-52.

Bremner, J.M. and Jenkinson, D.S. 1960 Determination of organic carbon in soil. I. Oxiation by dichromate of organic matter in soil and plant residues. J. Soil Sci. 11 394-402.

Bremner, J.M. 1965 Inorganic Nitrogen. In Methods of Soil Analysis (Part 2). Ed. Black, C.A. Amer. Soc. Agron. 1149-1178.

Bremner, J.M. 1965 Nitrogen availability indexes. In Methods of Soil Analysis (Part 2). Ed. Black, C.A. Amer. Soc. Agron. 1324-1345.

Bremner, J.M. and Blackmer, A.M. 1978 Nitrous oxide: Emission from soils during nitrification of fertiliser nitrogen. Science 199 295-296.

Bremner, J.M. and Blackmer, A.M. 1979 Effects of acetylene and soil water content on emission of nitrous oxide from soils. Nature 280 380-381.

Broadbent, F.E. 1951 Denitrification in some California soils. Soil Sci. 72 129-137.

Buckingham, E. 1904 Contributions to our knowledge of the aeration of soils. U.S.D.A. Bureau of Soils. Bulletin 25.

Bulla, L.A.Jr., Gilmoor, C.M. and Bollen, W.B. 1968 Enzymatic versus non-enzymatic denitrification in soil. Bacteriol. Proc. 4

Bulla, L.A.Jr., Gilmour, C.M. and Bollen, W.B. 1970 Non-biological reduction of nitrite in soil. Nature 225 664.

Burford, J.R. and Millington, R.J. 1968 Nitrous oxide in the atmosphere of a red-brown earth. Trans. 9th Intr. Congr. of Soil Sci. 2 505-515.

Burford, J.R. and Stefanson, R.C. 1973 Measurement of gaseous losses of nitrogen from soils. Soil Biol. Biochem. 5 133-141.

Burford, J.R., Ayanabe, A., Lal, R. and Greenland, D.J. 1975 Denitrification in a well drained tropical soil. Letcombe Lab. Ann. Report, Agric. Res. Counc., Wantage, England, 54.

Burford, J.R. and Bremner, J.M. 1975 Relationships between the denitrification capacities of soils and total, water soluble, and readily decomposable soil organic matter. Soil Biol. Biochem. 7 389-394.

Burford, J.R. 1976 Effect of the application of cow slurry to grassland on the composition of the soil atmosphere. J. Sci. Food Agric. 27 115-126.

Burford, J.R., Dowdell, R.J. and Lynch, J.M. 1978 Denitrification in British soils. In Nitrogen and the Environment (Vol. 2). Eds. Nielsen, D.R., Macdonald, J.G. Academic Press Inc. New York 365-377.

Burford, J.R., Dowdell, R.J., Crees, R. and Hall, K.C. 1978 Soil aeration and denitrification. Letcombe Lab. Ann. Report, Agric. Res. Counc., Wantage, England, 26.

Burford, J.R., Dowdell, R.J. and Crees, R. 1981 Emission of nitrous oxide to the atmosphere from direct drilled and ploughed clay soils. J. Sci. Food Agric. 32 219-223.

Cady, F.B. and Bartholomew, W.V. 1960 Sequential products of anaerobic denitrification in Norfolk soil material. Soil Sci. Soc. Amer. Proc. 24] 477-482.

Calder, K., Burke, K.A. and Lascelles, J. 1980 Introduction of nitrate reductase and membrane cytochromes in wild type and chlorate-resistant Paracoccus denitrificans. Arch. Microbiol. 126 149-153.

CAST 1976 Effect of increased nitrogen fixation on stratospheric ozone. Council for Agricultural Science and Technology Report No. 53. Iowa State Univ., Ames, Iowa.

Catroux, G. 1981 Effect of animal manures on organic matter and nitrogen contents of soils - a short review. In Nitrogen Losses and Surface Run-off from Landspreading of manures. Ed. Brogan, J.C. Martinus Nijhoff/Dr W. Junk. 349-366.

Cawse, P.A. and Sheldon, D. 1972. Rapid reduction of nitrate in soil remoistened after air drying. J. Agric. Sci. 78 405-412.

Chapman, H.D. and Pratt, P.F. 1961 Methods of Analysis for soils, plants and water. Univ. of California, Div. of Agric. Sci. 1961.

Chatelain, R. 1969 Reduction du nitrite par Alcaligenes odorans var. viridans. An. Inst. Pasteur 116 498-500.

Chichester, F.W. 1978 Disposition of ^{15}N labelled fertiliser nitrate applied during corn culture in a field lysimeter. J. Environ. Qual. 7 227-232.

Cho, C.M. and Sakdinan, L. 1978 Mass spectrometric investigation on denitrification. Can. J. Soil Sci. 58 443-457.

Cho, C.M. 1982 Oxygen consumption and denitrification kinetics in soil. Soil Sci. Soc. Amer. Proc. 46 756-762.

Christensen, S. 1980 Percolation studies of denitrification. Acta Agric. Scand. 30 225-236.

Chung, C.W. and Najjar, V.A. 1956 Cofactor requirements for enzymatic denitrification I Nitrite reductase. J. Biol. Chem. 218 617-625.

Clark, L.C.Jr., Wolf, R., Granger, D. and Taylor, Z. 1953 Continuous recording of blood oxygen tensions by polarography. J. Appl. Physiol. 6 189-193.

Cochran, V.L., Elliott, L.F. and Papendick, R.I. 1981 Nitrous oxide emissions from a fallow field fertilised with anhydrous ammonia. Soil Sci. Soc. Am. J. 45 307-310.

Colbourn, P. and Harper, I.W. 1982 Denitrification losses from an undrained direct-drilled clay soil. Letcombe Lab. Ann. Report, Agric. Res. Counc., Wantage, England. 48-50.

Colbourn, P., Hall, K.C. and Chapman, S.J. 1982 The stimulation of denitrification following C_2H_2 metabolism by soil micro-organisms. Letcombe Lab. Ann. Report, Agric. Res. Counc., Wantage, England. 50-51.

Conrad, R. and Seiler, W. 1979 Field measurement of hydrogen evolution by nitrogen fixing legumes. Soil Biol. and Biochem. 11 689-690.

Cooper, G.S. and Smith, R.L. 1963 Sequence of products formed during denitrification in some diverse western soils. Soil Sci. Soc. Amer. Proc. 27 659-662.

Cox, C.D., Payne, W.J. and Dervartanian, D.V. 1971 Electron paramagnetic resonance studies on the nature of hemeproteins in nitrite and nitric oxide reduction. Biochim. Biophys. Acta 253 290-294.

Cox, C.D.Jr., and Payne, W.J. 1973 Separation of soluble denitrifying enzymes and cytochromes from Pseudomonas perfectomarinus. Can. J. Microbiol. 19 861-862.

Crank, J. 1975 The mathematics of diffusion. Oxford University Press (2nd Edition).

Cranston, J.A. and Lloyd, B. 1930 Experiments on bacterial denitrification. J.R. Tech. Coll. (Glasgow) 2 301-315.

Craswell, E.T. and Martin, A.E. 1974 Effect of moisture content on denitrification. Soil Biol. Biochem. 6 127-129.

Craswell, E.T. 1979 Isotopic studies of the nitrogen balance in a cracking clay. IV. Fate of 3 nitrogen fertilisers in fallow soil in the field. Aust. J. Soil Res. 17 317-323.

Cribbs, W.H. and Mills, H.A. 1979 Influence of nitapyrin on the evolution of nitrous oxide from an organic medium with or without plants. Commun. in Soil Sci. and Plant Anal. 10 785-794.

Crooke, W.M. and Simpson, W.E. 1971 Determination of ammonia in Kjeldahl digests of crops by an automated procedure. J. Sci. Food Agric. 22 9-10.

Crutzen, P.J. 1974 Estimates of possible variations in total ozone due to natural causes and human activities. Ambio 3 201-210.

Culbertson, C.W., Zehnder, A.J.B. and Oremland, R.S. 1981 Anaerobic oxidation of acetylene by estuarine sediments and enrichment cultures. Appl. Environ. Microbiol. 41 396-403.

Currie, J.A. 1960 Gaseous diffusion in porous media: I Br. J. Appl. Phys. 11 314-317.

Currie, J.A. 1960 Gaseous diffusion in porous media: II Br. J. Appl. Phys. 11 318-324.

Currie, J.A. 1961 Gaseous diffusion in the aeration of aggregated soils. Soil Sci. 92 40-45.

Currie, J.A. 1965 Diffusion within soil microstructure. A structural parameter for soils. J. Soil Sci. 16 279-289.

Dam Kofoed, A. 1981 Crop uptake of nitrogen. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Ed. Brogan, J.C. Martinus Nijhoff/Dr W. Junk. 125-160.

De Bont, J.A.M. 1975 Oxidation of ethylene by bacteria. Ann. Appl. Biol. 81 119-121.

De Bont, J.A.M. 1976 Bacterial degradation of ethylene and the acetylene reduction test. Can. J. Microbiol. 22 1060-1062.

De Bont, J.A.M. and Mulder, E.G. 1976 Invalidity of the acetylene reduction assay in alkane-utilising nitrogen-fixing bacteria. Appl. Environ. Microbiol. 31 640-647.

De Bont, J.A.M. and Peck, M.W. 1980 Metabolism of acetylene by Rhodococcus A.I. Arch. Microbiol. 127 99-104.

Delwiche, C.C. 1959 Production and utilisation of nitrous oxide by Pseudomonas denitrificans. J. Bacteriol. 77 55-59.

Denmead, O.T. 1979 Chamber systems for measuring N_2O emission from soils in the field. Soil Sci. Soc. Amer. J. 43 89-95.

Denmead, O.T., Freney, J.R. and Simpson, J.R. 1979 Nitrous oxide emission during denitrification in a flooded field. Soil Sci. Soc. Amer. J. 43 716-719.

Denmead, O.T., Freney, J.R. and Simpson, J.R. 1979 Studies of nitrous oxide emission from a grass sward. Soil Sci. Soc. Amer. J. 43 726-728.

Dilkova, R. and Galeva, V. 1978 Aeration characteristics in a light gray forest gley soil. Pochvovedenie 7 168-172.

Dilworth, M.J. 1966 Acetylene reduction by nitrogen-fixing preparations from Clostridium pasteurianum. Biophys. Acta 127 285-294.

Domby, W. and Kohnke, H. 1956 The influence of soil crusts on gaseous diffusion. Soil Sci. Soc. Amer. Proc. 20 1-5.

Doner, H.E., Volz, M.G., Belser, L.W. and Løken, J.P. 1975 Short term nitrate loss and associated microbial populations in soil columns. Soil Biol. Biochem. 7 261-263.

Dowdell, R.J., Smith, K.A., Crees, R. and Restall, S.W.F. 1972 Field studies of ethylene in the soil atmosphere - equipment and preliminary results. Soil Biol. Biochem. 4 325-331.

Dowdell, R.J. and Smith, K.A. 1974 Field studies of the soil atmosphere. II. Occurrence of nitrous oxide. J. Soil Sci. 25 231-238.

Dowdell, R.J. and Crees, R. 1974 Measurements of nitrous oxide content of the atmosphere. Lab. Practice 23 488-489.

Dowdell, R.J. and Webster, C.P. 1976 Denitrification and leaching of nitrogen fertilisers. In Agriculture and Water Quality. MAFFS Tech. Bull. 32 163-173.

Dowdell, R.J., Crees, R., Burford, J.R., Cannell, R.Q. 1979 Oxygen concentrations in a clay soil after ploughing or direct drilling. J. Soil Sci. 30 239-245.

Dowdell, R.J., Burford, J.R. and Crees, R. 1979 Losses of nitrous oxide in drainage water from agricultural land. Nature 278 342-343.

Dowdell, R.J. 1982 Fate of nitrogen applied to agricultural crops with particular reference to denitrification. Phil. Trans. R. Soc. (London) 296B 363-373.

Dubey, H.D. and Fox, R.H. 1974 Denitrification losses from humid tropical soils of Puerto Rico. Soil Sci. Soc. Amer. Proc. 38 917-920.

Ekpete, D.M. and Cornfield, A.H. 1964 Losses, through denitrification, from soil of applied inorganic nitrogen even at low moisture contents. Nature 201 322-323.

Epstein, E. and Kohnke, H. 1957 Soil aeration as affected by organic matter application. Soil Sci. Soc. Amer. Proc. 21 585-588.

Eskew, D.L., Focht, D.D. and Ting, I.P. 1977 * see below Appl. Environ. Microbiol. 34 582-585.

Farrell, D.A., Greacen, E.L. and Gurr, C.G. 1966 Vapour transfer in soil due to air turbulence. Soil Sci. 102 305-313.

Federova, R.I., Milekhina, E.I. and Il'yukhina, N.I. 1973 Possibility of using the "gas exchange" method to detect extraterrestrial life. Identification of nitrogen fixing organisms. Akad. Nauk SSR Izvestia Ser. Biol. 6 797-806.

Fick, A. 1855 Über Diffusion. Annal. Phys. (Leipzig) 170 59-86.

Flühler, H., Ardakani, M.S., Szuskiewicz, T.E. and Stolzy, L.H. 1976 Field measured nitrous oxide concentrations, redox potentials, oxygen diffusion rates and oxygen partial pressures in relation to denitrification. Soil Sci. 122 107-114.

Flühler, H., Stolzy, L.H. and Ardakani, M.S. 1976 A statistical approach to define soil aeration in respect to denitrification. Soil Sci. 122 115-123.

Findlay, W.I. and McKenney, D.J. 1979 Direct measurement of nitrous oxide flux from soil. Can. J. Soil Sci. 59 413-421.

Focht, D.D. and Joseph, H. 1973 An improved method for the enumeration of denitrifying bacteria. Soil Sci. Soc. Amer. Proc. 37 698-699.

Focht, D.D. 1978 Methods for analysis of denitrification in soils. In Nitrogen and the Environment Vol. 2. Ed. Nielsen, D.R. Academic Press, New York 433-490.

Focht, D.D. and Stolzy, L.H. 1978 Long term denitrification studies in soils fertilised with $^{15}(NH_4)_2SO_4$. Soil Sci. Soc. Amer. J. 42 894-898.

Focht, D.D., Stolzy, L.H. and Meek, B.D. 1979 Sequential reduction of nitrate and nitrous oxide under field conditions as brought about by organic amendments and irrigation management. Soil Biol. Biochem. 11 37-46.

Forget, P. and Der Vartanian, D.V. 1972 The bacterial nitrate reductases: E.P.R. studies on nitrate reductase A from Micrococcus denitrificans. Biochim. Biophys. Acta 256 600-606.

Freney, J.R., Denmead, O.T. and Simpson, J.R. 1978 Soil as a source or sink for atmospheric nitrous oxide. Nature 273 530-532.

* Nitrogen fixation, denitrification, and pleomorphic growth in a highly pigmented Spirillum lipoferum

Freney, J.R., Denmead, O.T. and Simpson, J.R. 1979 Nitrous oxide emission from soils at low moisture contents. Soil Biol. Biochem. 11 167-173.

Furr, J.R. and Aldrich, W.W. 1943 Oxygen and carbon dioxide changes in the soil atmosphere of an irrigated date garden on calcareous very fine sandy loam. Proc. Amer. Soc. Hort. Sci. 42 46-52.

Gamble, T.N., Betlach, M.R. and Tiedje, J.M. 1977 Numerically dominant denitrifying bacteria from world soils. Appl. Environ. Microbiol. 33 926-939.

Garcia, J.L. 1974 Reduction de l'oxyde nitreux dans les sols de rizieres du senegal: mesure de l'activite denitrifiante. Soil Biol. Biochem. 6 79-84.

Garcia, J.L. 1977 Etude de la dénitrification chez une bactérie thermophile sporulée. Ann. Microbiol. (Inst. Pasteur) 128A 447-458.

Garcia, J.L., Pichinoty, F., Mandel, M. and Greenway, B. 1977 A new denitrifying saprophyte related to Pseudomonas pickettii. Ann. Microbiol. (Inst. Pasteur) 128A 229-237.

Gaskell, J.F., Blackmer, A.M. and Bremner, J.M. 1981 Comparison of effects of nitrate, nitrite, and nitric oxide on reduction of nitrous oxide to dinitrogen by soil microorganisms. Soil Sci. Soc. Amer. J. 45 1126-1127.

Gayon, U. and Dupetit, G. 1882 Sur la fermentation des nitrates. C.R. Acad. Sci. 95 644-646.

Germon, J.C. 1980 Etude quantitative de la dénitrification biologique dans le sol a l'aide de l'acétylène. I. Application à differents sols. Ann. Microbiol. (Inst. Pasteur) 131B 69-80.

Germon, J.C. 1980 Etude quantitative de la dénitrification biologique dans le sol à l'aide de l'acétylène. II. Evolution de l'éffet inhibiteur de l'acétylène sur la N_2O-réductase; incidence le l'acétylène sur la vitesse de dénitrification et sur la réorganisation de l'azote nitrique. Ann. Microbiol. (Inst. Pasteur) 131B 81-90.

Germon, J.C., Giraud, J.J., Chaussod, R. and Duthion, C. 1979 Nitrogen mineralisation and nitrification of pig slurry added to soil in laboratory conditions. In Modelling N from Farm Wastes. Ed. Gasser, J.K.R. Appl. Sci. Pub. 170-184.

Germon, J.C. and Couton, Y. 1981 Effects of repeated landspreadings of pig slurry on the nitrifying and denitrifying activities in soils. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Ed. Brogan, J.C., Martinus Nijhoff/Dr W. Junk. 416-424.

Gilbert, R.G., Lance, J.C. and Miller, J.B. 1979 Denitrifying bacteria populations and nitrogen removal in soil columns intermittently flooded with secondary sewage effluent. J. Environ. Qual. 8 101-104.

Gilliam, J.W., Dassberg, S., Lund, L.J. and Focht, D.D. 1978 Denitrification in four California soils: Effect of soil profile characteristics. Soil Sci. Soc. Amer. J. 42 61-66.

Goreau, T.J., Kaplan, W.A., Wofsy, S.C., McElroy, M.B., Valois, F.W. and Watson, S.W. 1980 Production of nitrite and nitrous oxide by nitrifying bacteria at reduced concentrations of oxygen. Appl. Environ. Microbiol. 40 526-532.

Grable, A.R. and Siemer, E.G. 1968 Effects of bulk density, aggregate size and soil water suction on oxygen diffusion, redox potential and elongation of corn roots. Soil Sci. Soc. Amer. Proc. 32 180-186.

Greenberg, E.P. and Becker, G.E. 1977 Nitrous oxide as end product of denitrification by strains of fluorescent pseudomonads. Can. J. Microbiol. 23 903-907.

Greenland, D.J. 1962 Denitrification in some tropical soils. J. Agric. Sci. 58 227-233.

Greenwood, D.J. 1961 The effect of oxygen concentration on the decomposition of organic materials in soil. Plant Soil 14 360-376.

Greenwood, D.J. and Berry, G. 1962 Aerobic respiration in soil crumbs. Nature 195 161-163.

Greenwood, D.J. 1963 Nitrogen transformations and the distribution of oxygen in soil. Chem. Ind. 17 799-803.

Greenwood, D.J. and Goodman, D. 1967 Direct measurement of the distribution of oxygen in soil aggregates and in columns of fine crumbs. J. Soil Sci. 18 182-196.

Guenzi, W.D., Beard, W.E., Watanabe, F.S., Olsen, S.R. and Porter, L.K. 1978 Nitrification and denitrification in cattle manure-amended soil. J. Environ. Qual. 7 196-202.

Guthrie, T.F. and Duxbury, J.M. 1978 Nitrogen mineralisation and denitrification in organic soils. Soil Sci. Soc. Amer. J. 42 908-912.

Hall, K.C. and Burford, J.R. 1975 Detection of nitrous oxide at ambient atmospheric concentrations. Letcombe Lab. Ann. Report., Agric. Res. Counc., Wantage, England 55.

Hauck, R.D. and Melsted, S.W. 1956 Some aspects of the problems of evaluating denitrification in soils. Soil Sci. Soc. Amer. Proc. 20 361-364.

Harpstead, M.I. and Brage, B.L. 1958 Storage of soil samples and its effect on the subsequent accumulation of nitrate nitrogen during controlled incubation. Soil Sci. Soc. Amer. Proc. 22 326-334.

Henriksen, A. and Selmer-Olsen, A.R. 1970 Automatic methods for determining nitrate and nitrite in water and soil extracts. Analyst 95 514-518.

Hill, A.R. 1979 Denitrification in the nitrogen budget of a river ecosystem. Nature 281 291-292.

Hooper, A.L. and Terry, K.R. 1973 Specific inhibitors of ammonia oxidation in Nitrosomonas. J. Bacteriol. 115 480-485.

Hutchinson, G.L. and Mosier, A.R. 1979 Nitrous oxide emissions from an irrigated cornfield. Science 205 1125-1127.

Hutchinson, G.L. and Mosier, A.R. 1981 Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Amer. J. 45 311-316.

Hynes, R.K. and Knowles, R. 1978 Inhibition by acetylene of ammonia oxidation in Nitrosomonas europaea. FEMS Microbiol. Lett. 4 319-321.

Hynes, R.K. and Knowles, R. 1980 Denitrification, nitrogen fixation and nitrification in continuous flow laboratory soil columns. Can. J. Soil Sci. 60 355-363.

Iwasaki, H. and Mori, T. 1958 Studies on denitrification. III. Enzymatic gas production by the reaction of nitrite with hydroxylamine. J. Biochem. (Tokyo) 45 133-140.

Iwasaki, H., Shidara, S., Suzuki, H. and Mori, T. 1963 Studies on denitrification. VII. Further purification and properties of denitrifying enzyme. J. Biochem. (Tokyo) 53 299-303.

Jackson, M.L. 1958 Soil Chemical Analysis. Constable and Co.

Jacobson, S.N. and Alexander, M. 1980 Nitrate loss from soil in relation to temperature, carbon source and denitrifier populations. Soil Biol. Biochem. 12 501-505.

Jansson, S.L. and Clarke, F.E. 1952 Losses of nitrogen during decomposition of plant material in the presence of inorganic nitrogen. Proc. Soil Sci. Soc. Amer. 16 330-334.

Jolley, V.D. and Pierre, W.H. 1977 Profile accumulation of fertiliser derived nitrate and total nitrogen recovery in two long-term nitrogen-rate experiments with corn. Soil Sci. Soc. Amer. J. 41 373-378.

Jordan, J.H.Jr., Patrick, W.H.Jr. and Willis, W.H. 1967 Nitrate reduction by bacteria isolated from waterlogged Crowley soil. Soil Sci. 104 129-133.

Kamp-Nielsen, L. and Anderson, J.M. 1977 A review of the literature on sediment-water exchange of nitrogen compounds. Prog. Water Technol. 8 393-418.

Kanemasu, E.T., Powers, W.L. and Sij, J.W. 1974 Field chamber measurements of carbon dioxide flux from soil surface. Soil Sci. 118 233-237.

Kanner, D. and Bartha, R. 1979 Growth of Nocardia rhodochrous on acetylene gas. J. Bacteriol. 139 225-230.

Kanner, D. and Bartha, R. 1982 Metabolism of acetylene by Nocardia rhodochrous. J. Bacteriol. 150 989-992.

Keeney, D.R., Fillery, I.R. and Marx, G.P. 1979 Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. Soil Sci. Soc. Amer. J. 43 1124-1128.

Kempers, A.J. 1974 Determination of sub-micro quantities of ammonium and nitrates in soils with phenol, sodium nitroprusside and hypochlorite. Geoderma 12 201-206.

Khan, M.F.A. and Moore, A.W. 1968 Denitrifying capacity of some Alberta soils. Can. J. Soil Sci. 48 89-91.

Kiely, P.V. 1981 Gaseous nitrogen losses from slurry. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Ed. Brogan, J.C., Martinus Nijhoff/Dr W. Junk. 412-415.

Koike, I. and Hattori, A. 1975 Growth yield of a denitrifying bacterium, Pseudomonas denitrificans, under aerobic and denitrifying conditions. J. Gen. Microbiol. 88 1-10.

Kim, C.M. 1973 Influence of vegetation types on the intensity of ammonia and nitrogen dioxide liberation from soil. Soil Biol. Biochem. 5 163-166.

Kimball, B.A. and Lemon, E.R. 1971 Air turbulence effects upon soil gas exchange. Soil Sci. Soc. Amer. Proc. 35 16-21.

Kimball, B.A. 1978 Critique on the application of gaseous diffusion theory to the measurement of denitrification. In Nitrogen in the Environemnt, (Vol. 1). Eds. Nielson, D.R. and MacDonald, J.G. Academic Press Ind., New York 351-361.

King, J.A. 1982 Aeration of upland soils under afforestation. Ph.D. Thesis. Edinburgh University.

Klemedtsson, L., Svenson, P.H., Lindberg, T. and Rosswall, T. 1977 The use of acetylene inhibition of nitrous oxide reductase in quantifying denitrification in soils. Swed. J. Agric. Sci. 7 179-185.

Kluyver, A.J. and Verhoeven, W. 1954 Studies on true dissimilatory nitrate reduction. II. The mechanism of denitrification. Antonie van Leeuwenhoek, J. Microbiol. Serol. 20 241-262.

Knowles, R. 1979 Denitrification, acetylene reduction and methane metabolism in Lake Sediment exposed to acetylene. Appl. Environ. Microbiol. 38 486-493.

Kohl, D.H., Vithayathil, F., Whitlow, P., Shearer, G. and Shien, S.H. 1976 Denitrification kinetics in soil systems: the significance of good fit of data to mathematical forms. Soil Sci. Soc. Amer. J. 40 249-253.

Koike, I. and Hattori, A. 1975 Energy yield of denitrification: an estimate from growth yield in continuous cultures of Pseudomonas denitrificans under nitrate-, nitrite-, and nitrous oxide-limited conditions. J. Gen. Microbiol. 88 11-19.

Kolenbrander, G.J. 1981 Leaching of nitrogen in agriculture. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Ed. Brogan, J.C. Martinus Nijhoff/Dr W. Junk. 199-216.

Kolenbrander, G.J. 1981 Mineralisation of nitrogen as influenced by decomposition rate of soil organic matter. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Ed. Brogan, J.C. Martinus Nijhoff/Dr W. Junk. 380-383.

Koskinen, W.C. and Keeney, D.R. 1982 Effect of pH on the rate of gaseous products of denitrification in a silt loam soil. Soil Sci. Soc. Amer. J. 46 1165-1167.

Kowalenko, C.G. and Cameron, D.R. 1978 Nitrogen transformations in soil-plant systems in three years of field experiments using tracer and non-tracer methods on an ammonium fixing soil. Can. J. Soil Sci. 58 195-208.

Kristjansson, J.K. and Hollocher, T.C. 1980 First practical assay for soluble nitrous oxide reductase of denitrifying bacteria and a partial kinetic characterization. J. Biol. Chem. 255 704-707.

Lai, S.H., Tiedje, J.M. and Erickson, A.E. 1976 In situ measurement of gas diffusion coefficient in soil. Soil Sci. Soc. Amer. J. 40 3-6.

Leffelaar, R.A. 1979 Simulation of partial anaerobiosis in a model soil in respect to denitrification. Soil Sci. 128 110-120.

Lemon, E.R. and Erickson, A.E. 1952 The measurement of oxygen diffusion in the soil with a platinum micro-electrode. Soil Sci. Soc. Amer. Proc. 16 160-163.

Lensi, R. and Chalamet, A. 1979 Relations nitrate-oxyde nitreux lors de la dénitrification dans un sol hydromorphe. Revue d'Ecologie et du Biologie du Sol 16 315-323.

Letey, J., Stolzy, L.H., Valoras, N. and Szuszkiewicz, T.E. 1962 Influence of soil oxygen on growth and mineral concentrations of barley. Agron. J. 54 538-540.

Letey, J., Jury, W.A., Hadas, Aviva and Valoras, N. 1980 Gas diffusion as a factor in laboratory incubation studies on denitrification. J. Environ. Qual. 9 223-227.

Letey, J., Valoras, N., Hadas, Aviva and Focht, D.D. 1980 Effect of air filled porosity, nitrate concentration, and time on the ratio of nitrous oxide to nitrogen evolution during denitrification. J. Environ. Qual. 9 227-231.

Letey, J., Hadas, Aviva, Valoras, N. and Focht, D.D. 1980 Effect of preincubation treatments on the ratio of nitrous oxide to nitrogen evolution. J. Environ. Qual. 9 232-235.

Letey, J., Valoras, N., Focht, D.D. and Ryden, J.C. 1981 Nitrous oxide production and reduction during denitrification as affected by redox potential. Soil Sci. Soc. Amer. J. 45 727-730.

Limmer, A.W., Steele, K.W. and Wilson, A.T. 1982 Direct field measurement of nitrogen and nitrous oxide evolution from soil. J. Soil Sci. 33 499-507.

Lind, A.M. 1980 Denitrification in the root zone. Tidsskrift for Planteavl. 84 101-110.

Liu, S.C., Cicerone, R.J. and Donahue, T.M. 1977 Sources and sinks of atmospheric nitrous oxide and the possible ozone reduction due to industrially fixed nitrogen fertilisers. Tellus 29 251-263.

Macgregor, A.N. 1972 Gaseous losses of nitrogen from freshly wetted desert soils. Soil Sci. Soc. Amer. Proc. 36 594-596.

Mahendrappa, M.K. and Smith, R.L. 1967 Some effects of moisture on denitrification in acid and alkaline soils. Soil Sci. Soc. Amer. Proc. 31 212-215.

Mann, L.D., Focht, D.D., Joseph, H.A. and Stolzy, L.H. 1972 Increased denitrification in soils by additions of sulfur as an energy source. J. Environ. Qual. 1 329-332.

Matsubara, T. and Mori, T. 1968 Studies on denitrification. IX. Nitrous oxide, its production and reduction to nitrogen. J. Biochem. (Tokyo) 64 863-871.

Matsubara, T. 1975 The participation of cytochromes in the reduction of nitrous oxide to nitrogen by a denitrifying bacteria. J. Biochem. (Tokyo) 77 627-632.

Matthias, A.D., Yarger, D.N. and Weinbeck, R.S. 1978 A numerical evaluation of chamber methods for determining gas fluxes. Geophys. Res. Lett. 5 765-768.

Matthias, A.D., Blackmer, A.M. and Bremner, J.M. 1980 A simple chamber technique for field measurement of emission of nitrous oxide from soils. J. Environ. Qual. 9 251-256.

McAllister, J.S.V. 1977 Spreading slurry on land. J. Soil Sci. 123 338-343.

McElroy, M.B., Elkins, J.W., Wofsky, S.C. and Lung, Y.L. 1976 Sources and sinks for atmospheric nitrous oxide. Rev. Geophys. Space Phys. 14 143-150.

McElroy, M.B., Wofsky, S.C. and Lung, Y.L. 1977 The nitrogen cycle: perturbations due to man and their impact on atmospheric nitrous oxide and ozone. Philos. Trans. R. Soc. (London) 227B 159-181.

McGarity, J.W. 1962 Effect of freezing of soil on denitrification. Nature (London) 196 1342-1343.

McGarity, J.W. and Myers, R.J.K. 1968 Denitrifying activity in solodized solonetz soils of Eastern Australia. Soil Sci. Soc. Amer. Proc. 32 812-817.

McGarity, J.W. and Hauck, R.D. 1969 An aeromatric apparatus for the evaluation of gaseous nitrogen transformations in field soils. Soil Sci. 108 335-344.

McGarity, J.W. and Rajaratnam, J.A. 1973 Apparatus for the measurement of losses of nitrogen as gas from the field and simulated field environments. Soil Biol. Biochem. 5 121-131.

McIntyre, D.S. 1970 The platinum electrode method for soil aeration measurement. Adv. Agron. 22 235-283.

McKenna, E.J. and Kallis, R.E. 1965 The biology of hydrocarbons. Ann. Rev. Microbiol. 19 183-208.

McKenney, D.J., Shuttlework, K.F. and Findlay, W.I. 1980 Nitrous oxide evolution rates from fertilised soil: effect of applied nitrogen. Can. J. Soil Sci. 60 429-438.

McKenney, D.J., Shuttlework, K.F. and Findlay, W.I. 1980 Temperature dependence of nitrous oxide production from Brookston day. Can. J. Soil Sci. 60 665-674.

McKenzie, E. and Kurtz, L.T. 1976 Effect of pretreatment on loss of ^{15}N labelled fertiliser from waterlogged soil during incubation. Soil Sci. Soc. Amer. J. 40 534-537.

Meek, B.D., Grass, L.B. and Mackenzie, A.J. 1962 Applied nitrogen losses in relation to oxygen status of soils. Soil Sci. Soc. Amer. Proc. 33 575-578.

Millington, R.J. 1959 Gas diffusion in porous media. Science 130 100-102.

Misra, C., Nielsen, D.R. and Biggar, J.W. 1974 Nitrogen transformations in soil during leaching. I. Theoretical considerations. Soil Sci. Soc. Amer. Proc. 38 289-293.

Misra, C., Nielsen, D.R. and Biggar, J.W. 1974 Nitrogen transformations in soil during leaching. II. Steady state nitrification and nitrate reduction. Soil Sci. Soc. Amer. Proc. 38 294-299.

Miyata, M. and Mori, T. 1968 Studies on denitrification VIII Production of nitric oxide by denitrifying reaction in the presence of tetramethyl-p-Phenylenediamine. J. Biochem. (Tokyo) 64 849-861.

Mosier, A.R. and Hutchinson, G.L. 1981 Nitrous oxide emissions from cropped fields. J. Environ. Qual. 10 169-173.

Müller, M.M., Sundman, V. and Skujins, J. 1980 Denitrification in low pH spodosols and peats determined with the acetylene inhibition method. App. Environ. Microbiol. 40 235-239.

Myers, R.J.K. 1972 The effects of sulfide on nitrate reduction in soil. Plant Soil 37 431-433.

Myers, R.J.K. and McGarity, J.W. 1972 Denitrification in undisturbed cores from a solodized solonetz B horizon. Plant Soil 37 81-89.

Nelson, D.W., Bremner, J.M. 1970 Gaseous products of nitrite decomposition in soils. Soil Biol. Biochem. 2 203-215.

Neyra, C.A., Döbereiner, J., Lalande, R. and Knowles, R. 1977 Denitrification by nitrogen-fixing Spirillum lipoferum. Can. J. Microbiol. 23 300-305.

Niklewski, B. 1914 Quoted in Verhoeven et al. 1954.

Nommik, H. 1956 Investigations of denitrification in soil. Acta. Agric. Scand. 6 195-228.

Overrein, L.N. 1968 Lysimeter studies on tracer nitrogen in forest soil. I. Nitrogen losses by leaching and volatilization after addition of urea-^{15}N. Soil Sci. 106 280-290.

Papendick, R.I. and Runkles, J.R. 1965 Transient state oxygen diffusion in soil. I. A case when rate of oxygen consumption is constant. Soil Sci. 100 251-261.

Parkes, M.E. and O'Callaghan, J.R. 1977 Calculations for the efficient use of nutrients in livestock slurries on grassland. Adas. Qu. Rev. 27 155-167.

Patrick, W.H.Jr. 1960 Nitrate reduction rates in a submerged soil as affected by Redox Potential. Trans. Int. Congr. Soil Sci. 7th 2 494-500.

Patrick, W.H.Jr. and Tusneem, M.E. 1972 Nitrogen loss from flooded soil. Ecol. 53 735-737.

Patrick, W.H.Jr. and Gotoh, S. 1974 The role of oxygen in nitrogen loss from flooded soils. Soil Sci. 118 78-81.

Patriquin, D.G., MacKinnon, J.C. and Wilke, K.I. 1978 Seasonal pattern of denitrification activity and leaf nitrate reductase activity in a cornfield. Can. J. Soil Sci. 58 283-285.

Patten, D.K., Bremner, J.M. and Blackmer, A.M. 1980 Effects of drying and air dry storage of soils on their capacity for denitrification of nitrate. Soil Sci. Soc. Amer. J. 44 67-70.

Paul, E.A. and Victoria, R.L. 1978 Nitrogen transfer between the soil and the atmosphere. In Proceedings of the 3rd Symposium on Environmental Geochemistry. Ed. Krumbein, W. 525-541.

Payne, W.J., Riley, P.S. and Cox, C.D. 1971 Separate nitrite, nitric oxide, and nitrous oxide reducing fractions from Pseudomonas perfectomarinus. J. Bacteriol. 106 356-361.

Payne, W.J. 1976 Denitrification. Trends Biochem. Sci. 1 220-222.

Payne, W.J. 1981 Denitrification. Wiley. New York.

Payne, W.J. and Grant, M.A. 1981 Overview of denitrification. In Enhancing Biological Production of Ammonia from Atmospheric Nitrogen and Soil Nitrate. Ed. Lyons, J.M. and Rains, D.W. Plenum Publishing. New York.

Pearce, S.C. 1964 Biological Statistics: An Introduction. McGraw Hill. New York.

Penman, H.L. 1940 Gas and vapour movement in the soil. I. The diffusion of vapours through porous solids. J. Agric. Sci. 30 437-462.

Penman, H.L. 1940 Gas and vapour movements in the soil. II. The diffusion of carbon dioxide through porous solids. J. Agric. Sci. 30 470-481.

Pfitzner, J. and Schlegel, H.G. 1973 Denitrifikation bei Hydrogenomonas eutropha Stam H16. Arch. Mikrobiol. 90 199-211.

Pichinoty, F., Bigliardi-Rouvier, J., Mandel, M., Greenway, B., Metenier, G. and Garcia, J.L. 1976 The isolation and properties of a denitrifying bacterium of the genus Flavobacterium. Antonie van Leeuwenhoek 42 349-354.

Pichinoty, F., Mandel, M. and Garcia, J.L. 1977 Etude de six souches de Aerobacterium tumefaciens et A. Radiobacter. Ann. Microbiol. (Inst. Pasteur) 128A 303-310.

Pichinoty, F., Garcia, J.L., Mandel, M., Job, C. and Durand, M. 1978 Isolement de bacteries utilisant en anaerobiose l'oxyde nitrique comme accepteur d'electrons respiratoire. C.R. Acad. Sci. 286 1403-1405.

Pichinoty, F., Mandel, M. and Garcia, J.L. 1979 The properties of novel denitrifying Bacillus cultures found in tropical soils. J. Gen. Microbiol. 115 419-430.

Pilot, L. and Patrick, W.H.Jr. 1972 Nitrate reduction in soils: Effects of soil moisture tension. Soil Sci. 114 312-316.

Pomares-Garcia, F. and Pratt, P.F. 1978 Recovery of ^{15}N labelled fertiliser from manured and sludge amended soils. Soil Sci. Soc. Amer. J. 42 717-720.

Pratt, P.F., Davis, S. and Sharpless, R.G. 1976 A four-year trial with animal manures. II. Mineralisation of nitrogen. Hilgardia 44 113-125.

Pritchard, D.T. and Currie, J.A. 1982 Diffusion coeffients of carbon dioxide, nitrous oxide, ethylene and ethane in air and their measurement. J. Soil Sci. 33 175-184.

Radcliffe , B.C. and Nicholas, D.J.D. 1968 Some properties of a nitrite reductase from Pseudomonas denitrificans. Biochim. Biophys. Acta 153 545-554.

Ragg, J.M. and Futty, D.W. 1967 Soils of the Country round Haddington and Eyemouth. Memoirs of the Soil Survey of Gt. Britain and Scotland. Macaulay Institute for Soil Research. HMSO.

Raimboult, M. 1975 Etude de l'influence inhibitrice de l'acétylène sur la formation biologique de méthane dans un sol de rizière. Ann. Microbiol. (Inst. Pasteur) 126A 247-258.

Raney, W.A. 1950 Field measurement of oxygen diffusion through soil. Soil Sci. Soc. Amer. Proc. 14 61-65.

Reddy, K.R. and Patrick, W.H.Jr. 1975 Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. Soil Biol. Biochem. 7 87-94.

Reddy, K.R., Patrick, W.H.Jr. and Phillips, R.E. 1976 Ammonia diffusion as a factor in nitrogen loss from flooded soils. Soil Sci. Soc. Amer. J. 40 528-533.

Reddy, K.R., Patrick, W.H.Jr. and Phillips, R.E. 1978 The role of nitrate diffusion in determining the order and rate of denitrification in flooded soil: 1. Experimental results. Soil Sci. Soc. Amer. J. 42 268-272.

Reddy, K.R., Rao, P.S.C. and Jessup, R.E. 1982 The effect of carbon mineralisation on denitrification kinetics in mineral and organic soils. Soil Sci. Soc. Amer. J. 46 62-68.

Renner, E.D. and Becker, G.E. 1970 Production of nitric oxide and nitrous oxide during denitrification by Corynebacterium nephredii. J. Bacteriol. 101 821-826.

Reuss, J.O. and Smith, R.L. 1965 Chemical reduction of nitrites in acid soils. Soil Sci. Soc. Amer. Proc. 29 267-270.

Rigaud, J.F., Bergersen, F.J., Turner, G.L. and Daniel, R.M. 1973 Nitrate dependent anaerobic acetylene-reduction and nitrogen fixation by soybean bacteroids. J. Gen. Microbiol. 77 137-144.

Ritchie, G.A.F. and Nicholas, D.J.D. 1972 Identification of the sources of nitrous oxide produced by oxidative and reductive processes in Nitrosomonas europaea. Biochem. J. 126 1181-1191.

Robinson, F.E. 1957 A diffusion chamber for studying soil atmosphere. Soil Sci. 83 465-469.

Rolston, D.E., Fried, M. and Goldhamer, D.A. 1976 Denitrification measured directly from nitrogen and nitrous oxide fluxes. Soil Sci. Soc. Amer. J. 40 259-266.

Rolston, D.E. and Marino, M.A. 1976 Simultaneous transport of nitrate and gaseous denitrification products in soil. Soil Sci. Soc. Amer. J. 40 860-865.

Rolston, D.E. and Broadbent, F.E. 1977 Field measurement of denitrification. U.S. Environ. Protection Technol. Series. EPA-600/2-77-233.

Rolston, D.E. 1978 Application of gaseous-diffusion theory to measurement of denitrification. In Nitrogen in the Environment (Vol. 1). Ed. Nielsen, D.R. and MacDonald, J.G. Academic Press Inc. New York 309-335.

Rolston, D.E., Hoffman, D.L. and Toy, D.W. 1978 Field measurement of denitrification: 1. Flux of nitrogen and nitrous oxide. Soil Sci. Soc. Amer. J. 42 863-869.

Rolston, D.E., Broadbent, F.E. and Goldhamer, D.A. 1979 Field measurement of denitrification. II. Mass balance and sampling uncertainty. Soil Sci. Soc. Amer. J. 43 703-708.

Rolston, D.E., Sharpley, A.N., Toy, D.W., Hoffman, D.L. and Broadbent, F.E. 1980 Denitrification as affected by irrigation frequency of a field soil. U.S. Environ. Protection Agency EPA-600/2-80-066.

Rommel, L.G. 1922 Luftväxlingen i marken som ekologik factor. Meddel. Statens Skogsfarsöks anst. 19 125-359.

Russel, E.J. and Appleyard, A. 1915 The atmosphere of the soil, its composition and causes of variation. J. Agric. Sci. 7 1-48.

Ryden, J.C., Lund, L.J. and Focht, D.D. 1978 Direct in field measurement of nitrous oxide flux from soils. Soil Sci. Soc. Amer. Proc. 42 731-737.

Ryden, J.C., Lund, L.J. and Focht, D.D. 1979 Direct measurement of denitrification from soils: I. Laboratory evaluation of acetylene inhibition of nitrous oxide reduction. Soil Sci. Soc. Amer. J. 48 104-109.

Ryden, J.C., Lund, L.J., Letey, J. and Focht, D.D. 1979 Direct measurement of denitrification loss from soils: II. Development and application of field methods. Soil Sci. Soc. Amer. J. 43 110-118.

Ryden, J.C. and Lund, L.J. 1980 Nature and extent of directly measured denitrification losses from some irrigated vegetable crop production units. Soil Sci. Soc. Amer. J. 44 505-511.

Ryden, J.C. and Lund, L.J. 1980 Nitrous oxide evolution from irrigated land. J. Environ. Qual. 9 387-393.

Ryden, J.C. 1982 Effects of acetylene on nitrification and denitrification in two soils during incubation with ammonium nitrate. J. Soil Sci. 33 263-270.

Ryden, J.C. and Dawson, K.P. 1982 Evaluation of the acetylene-inhibition technique for the measurement of denitrification in grassland soils. J. Sci. Food Agric. 33 1 -10.

Ryden, J.C. 1983 Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. J. Soil Sci. 34 355-365.

Ryden, J.C. and Rolston, D.E. 1983 (in press) The measurement of Denitrification. In Gaseous Loss of Nitrogen from Plant-Soil Systems. Ed. Freney, J.R. and Simpson, J.R. Nijhoff.

Sacks, L.E. and Barker, H.A. 1952 Substrate oxidation and nitrous oxide utilisation in denitrification. J. Bacteriol. 64 247-252.

Sanders, L. 1980 Nitrogen balance experiments under forage maize and grass. J. Sci. Food Agric. 31 846-847.

Schaefer, R. 1964 [Effect of temperature, especially of repeated freezing, on dissimulative reduction of nitrates during incubation of a calcic mull and hydromull]. Ann. Inst. Pasteur 107 282-292.

Schwartzbeck, R.A., MacGregor, J.M. and Schmidt, E.L. 1961 Gaseous nitrogen losses from nitrogen fertilised soils measured with infra-red and mass spectroscopy. Soil Sci. Soc. Amer. Proc. 25 186-189.

Scotter, D.R., Thurtell, G.W. and Raats, P.A.C. 1967 Dispersion resulting from sinusoidal gas flow in porous materials. Soil Sci. 104 306-308.

Shearer, R.C., Millington, R.J. and Quirk, J.P. 1966 Oxygen diffusion through sands in relation to capillary hysteresis. 2. Quasi-steady-state diffusion of oxygen through partially saturated soils. Soil Sci. 101 432-436.

Skerman, V.B.D. and Macrae, I.C. 1957 The influence of oxygen availability on the degree of nitrate reduction by Pseudomonas denitrificans. Can. J. Microbiol. 3 505-530.

Smid, A.E. and Beauchamp, E.G. 1976 Effects of temperature and organic matter on denitrification in soil. Can. J. Soil Sci. 56 385-391.

Smith, C.J. and Chalk, P.M. 1980 Gaseous nitrogen evolution during nitrification of ammonia fertiliser and nitrite transformations in soils. Soil Sci. Soc. Amer. J. 44 277-282.

Smith, K.A. 1977 Soil Aeration. Soil Sci. 123 284-291.

Smith, K.A. 1980 A model of the extent of anaerobic zones in aggregated soils, and its potential application to estimate of denitrification. J. Soil Sci. 31 263-277.

Smith, M.S., Firestone, Mary, K., Tiedje, J.M. 1978 The acetylene inhibition method for short term measurement of soil denitrification and its evaluation using nitrogen-13. Soil Sci. Soc. Amer. J. 42 611-615.

Smith, M.S. and Tiedje, J.M. 1979 The effects of roots on soil denitrification. Soil Sci. Soc. Amer. J. 43 951-955.

Smith, M.S. and Zimmerman, Karen, 1981 Nitrous oxide production by non-denitrifying soil nitrate reducers. Soil Sci. Soc. Amer. J. 45 865-871.

Sørensen, J. 1978 Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. Appl. Environ. Microbiol. 36 139-143.

Sørensen, J., Tiedje, J.M. and Firestone, R.B. 1980 Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying Pseudomonas fluorescens. Appl. Environ. Microbiol. 39 105-108.

Soulides, D.A. and Allison, F.E. 1961 Effect of drying and freezing soils on carbon dioxide production, available mineral nutrients, aggregation, and bacterial population. Soil Sci. 91 291-298.

Spallacci, P. 1981 Nitrogen uptake by crops manure with pig slurry. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Ed. Brogan, J.C. Martinus Nijhoff/Dr W. Junk. 178-180.

Stanford, G., Legg, J.O., Dzienia, S. and Simpson, E.C.Jr. 1975 Denitrification and associated nitrogen transformations in soils. Soil Sci. 120 147-152.

Stanford, G., Van Der Pol, R.A. and Dzienia, S. 1975 Denitrification rate in relation to total and extractable soil carbon. Soil Sci. Soc. Amer. Proc. 39 284-289.

Stanford, G., Dzienia, S. and Van Der Poll, R.A. 1975 Effect of temperature on denitrification rate in soils. Soil Sci. Soc. Amer. Proc. 39 867-870.

Starr, J.L., Broadbent, F.E. and Nielsen, D.R. 1974 Nitrogen transformations during continuous leaching. Soil Sci. Soc. Amer. Proc. 38 283-289.

Starr, J.L. and Parlange, J.Y. 1975 Non-linear denitrification kinetics with continuous flow in soil columns. Soil Sci. Soc. Amer. Proc. 29 875-880.

Stefanson, R.C. and Greenland, D.J. 1970 Measurement of nitrogen and nitrous oxide evolution from soil-plant systems using sealed growth chambers. Soil Sci. 109 203-206.

Stefanson, R.C. 1972 Soil denitrification in sealed soil plant systems. I. Effect of plants, soil water content and soil organic matter content. Plant Soil 37 113-128.

Stefanson, R.C. 1972 Soil denitrification in sealed soil plant systems. II. Effect of soil water contents and form of applied nitrogen. Plant Soil 37 129-140.

Stefanson, R.C. 1973 Evolution patterns of nitrous oxide and nitrogen in sealed soil plant systems. Soil Biol. Biochem. 5 167-169.

Stefanson, R.C. 1976 Denitrification from nitrogen fertiliser placed at various depths in the soil-plant system. Soil Sci. 121 353-363.

Stolzy, L.H. and Letey, J. 1964 Correlation of plant response to oxygen diffusion rates. Hilgardia 35 567-576.

Suzuki, S. 1912 Über die Enstehung der Stickoxyde in Denitrifiekations-Progress. I. Prüfung, Bestimmung und Verkommen der Stickoxyduls in den Gärungsgasen. Zentralbl. Bakteriol. Parasitenkd. II Abt. 31 27-49.

Tackett, J.L. 1968 Theory and application of gas chromatography in soil aeration research. Soil Sci. Soc. Amer. Proc. 32 346-350.

Tam. T.Y. and Knowles, R. 1979 Effects of sulfide and acetylene on nitrous oxide reduction by soil and by Pseudomonas aeruginasa. Can. J. Microbiol. 25 1133-1138.

Taylor, G.S. and Abrahams, J.H. 1953 A diffusion equilibrium method for obtaining soil gases under field conditions. Soil Sci. Soc. Amer. Proc. 17 201-206.

Taylor, S.A. 1949 Oxygen diffusion in porous media as a measure of soil aeration. Soil Sci. Soc. Amer. Proc. 14 55-61.

Terry, R.E. and Tate, R.L. 1980 The effect of nitrate on nitrous oxide reduction in organic soils and sediments. Soil Sci. Soc. Amer. Proc. 44 744-746.

Thijell, A.A. and Burford, J.R. 1975 Effects of the application of cow slurry to grassland on nitrate levels in soil and soil water contents. J. Sci. Food Agric. 26 1203-1213.

Thom, A.S. 1975 Momentum, mass, and heat exchange of plant communities. In Vegetation and the Atmosphere. Vol. 1. Principles. Ed. J.L. Monteith. Academic Press Inc. New York 57-109.

Tinsley, J. 1950 The determination of organic carbon in soils by dichromate mixtures. Trans. Int. Congr. Soil Sci. 4th 1 161-164.

Tiren, T., Thorin, J. and Nommik, H. 1976 Denitrification measurements in lakes. Acta. Agric. Scand. 26 175-184.

Tunney, H. 1981 Nitrogen uptake by crop-grassland. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Developments in Plant and Soil Sciences Vol. 2. Ed. Brogan, J.C., Martinus Nijhoff/Dr W. Junk 161-166.

Van Bavel, C.H.M. 1951 A soil aeration theory based on diffusion. Soil Sci. 72 33-46.

Van Bavel, C.H.M. 1965 Composition of soil atmosphere. In Methods of Soil Analysis (Part 1). Ed. Black, A. Amer. Soc. Agron. 315-318.

Van Cleemput, O. 1971 [Denitrification study in soil]. Pedologie 21 367-376.

Van Cleemput, O. and Patrick, W.H.Hr. 1974 Nitrate and nitrite reduction in flooded -irradiated soil under controlled pH and redox potential conditions. Soil Biol. Biochem. 6 85-88.

Van Cleemput, O., Patrick, W.H.Jr. and McIlhenny, R.C. 1975 Formation of chemical and biological denitrification products in flooded soil at controlled pH and redox potential. Soil Biol. Biochem. 7 329-332.

Van Gent-Ruijters, M.L.W., De Vries, W. and Stouthamer, A.H. 1975 Influence of nitrate on fermentation pattern, molar growth yield, and synthesis of cytochrome b in Propionibacterium pentasaceum. J. Gen. Microbiol. 88 36-48.

Verhoeven, W. 1952 Aerobic spore forming nitrate reducing bacteria. Ph.D. Thesis. Delft University.

Verhoeven, W. and Goos, J.J.C. 1954 Studies on true dissimilatory nitrate reduction. I. Fate of the hydrogen donator in bacterial nitrate reduction. Antonie van Leeuwenhoek 20 93-101.

Vetter, H. and Steffens, G. 1981 Gaseous N losses. In Nitrogen Losses and Surface Run-off from Landspreading of Manures. Developments in Plant and Soil Sciences Vol. 2. Ed. Brogan, J.C. Martinus Nijhoff/Dr W. Junk 409-411.

Volz, M.G., Belser, L.W., Ardakani, M.S. and McLaren, A.D. 1974 Nitrate reduction and associated microbial populations in a ponded Hanford sandy loam. J. Environ. Qual. 4 99-102.

Volz, M.G., Belser, L.W., Ardakani, M.S. and McLaren, A.D. 1975 Nitrate reduction and nitrite utilization by nitrifiers in an unsaturated Hanford sandy loam. J. Environ. Qual. 4 179-182.

Volz, M.G. and Starr, J.L. 1977 Nitrate dissimulation and population dynamics of denitrifying bacteria during short term continuous flow. Soil Sci. Soc. Amer. J. 41 891-896.

Walker, G.C. and Nicholas, D.J.D. 1961 Nitrite reductase from Pseudomonas aeruginosa. Biochim. Biophys. Acta 49 350-360.

Wallingford, G.W., Murphy, L.S., Powers, W.L. and Manges, H.L. 1975 Denitrification in soil treated with beef-feedlot manure. Comm. Soil Sci. Pl. Anal. 6 147-161.

Walter, H.M., Keeney, D.R. and Fillery, I.R. 1979 Inhibition of nitrification by acetylene. Soil Sci. Soc. Amer. J. 43 195-196.

Wang, W.C., Yung, Y.L., Lacis, A.A., Mo, T. and Hensen, J.E. 1976 Greenhouse effects due to man-made perturbations of trace gases. Science 194 685-690.

Warrington, R. 1897 Denitrification and farmyard manure. J.R. Agric. Soc. Eng. 8 577-607.

Watanabe, I. and De Guzman, M.R. 1980 The effect of nitrate on acetylene disappearance from anaerobic soil. Soil Biol. Biochem. 12 193-194.

Webster, C.P. and Dowdell, R.D. 1982 Nitrous oxide emission from permanent grass swards. J. Sci. Food Agric. 33 227-230.

Weller, K.R., Stenhouse, N.S. and Watts, H. 1974 Diffusion of gases in porous solids (I & II). Can. J. Chem. 52 2684-2700.

Wesseling, J. 1962 Some solutions of the steady state diffusion of carbon dioxide through soils. Neth. J. Agric. Sci. 10 109-117.

Wesseling, J. and Van Wijk, W.R. 1957 Land drainage in relation to soils and crops. I. Soil physical conditions in relation to drain depth. In Drainage of Agricultural Lands. Ed. Luthin, L.N. Amer. Soc. Agron. Madison, Wisconsin.

Westerman, R.L. and Tucker, T.C. 1978 Factors affecting denitrification in a Sonoran desert soil. Soil Sci. Soc. Amer. J. 42 596-599.

Whittenbury, R., Phillips, K.C. and Wilkinson, J.F. 1970 Enrichment isolation and some properties of methane-utilising bacteria. J. Gen. Microbiol. 61 205-218.

Wijler, J. and Delwiche, C.C. 1954 Investigations on the denitrifying process in soil. Plant Soil 5 155-169.

Willey, C.R. and Tanner, C.B. 1963 Membrane-covered electrode for measurement of oxygen concentration in soil. Soil Sci. Soc. Amer. Proc. 27 511-515.

Willey, C.R. 1974 Elimination of errors caused by condensation of water on a membrane-covered electrode. Soil Sci. 117 343-346.

Woldendorp, J.W. 1963 The influence of living plants on denitrification. Meded. Landbouwhogesch. Wageningen 63 1-100.

Wood, J.J. and Greenwood, D.J. 1971 Distribution of carbon dioxide and oxygen in the gas phase of aerobic soils. J. Soil Sci. 22 281-288.

Yamaguchi, M., Howard, F.D., Hughes, D.L. and Flocker, W.J. 1962 An improved technique for sampling and analysis of soil atmospheres. Soil Sci. Soc. Amer. J. 26 512-513.

Yeomans, Jane C. and Beauchamp, E.G. 1978 Limited inhibition of nitrous oxide reduction in soil in the presence of acetylene. Soil Biol. Biochem. 10 517-519.

Yeomans, Jane C. and Beauchamp, E.G. 1982 Acetylene as a possible substrate in the denitrification process. Can. J. Soil Sci. 62 139-144.

Yeomans, Jane C. and Beauchamp, E.G. 1982 Sulfur in acetylene inhibition of nitrous oxide reduction by soil microorganisms. Soil Sci. Soc. Amer. J. 46 75-77.

Yoshida, T. and Alexander, M. 1970 Nitrous oxide formation by Nitrosomonas europaea and heterotrophic microorganisms. Soil Sci. Soc. Amer. Proc. 34 880-882.

Yoshinari, T. and Knowles, R. 1976 Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Comm. 69 705-710.

Yoshinari, T., Hynes, R. and Knowles, R. 1977 Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen-fixation in soil. Soil Biol. Biochem. 9 177-183.

Youatt, J.B. 1954 Denitrification of nitrite by a species of Achromobacter. Nature (London) 173 826-827.

Zablotowicz, R.M., Eskew, D.L. and Focht, D.D. 1978 Denitrification in Rhizobium. Can. J. Microbiol. 24 757-760.

Zobell, C.E. 1950 Assimilation of hydrocarbons by microorganisms. Adv. Enzymol. 10 443-486.

Zumft, W.G. and Vega, J.M. 1979 Reduction of nitrite to nitrous oxide by a cytoplasmic membrane fraction from the marine denitrifier Pseudomonas perfectomarinus. Biochim. Biophys. Acta 458 484-499.

APPENDIX 1: METHOD OF COLLECTING SOIL ATMOSPHERE SAMPLES

The sampling probes used (Fig. A.1) were similar to those described by Dowdell et al. (1972) and were relatively cheap, and sufficiently robust to last several seasons.

The probes consisted of a sintered bronze cup (Sintered Products Ltd., Sutton in Ashfield, Notts., England) of pore size 5μm, connected to an outer tube of electrical conduit by a flange fitted with a neoprene gasket. A stainless steel sampling tube was soldered through the flange to ensure a seal, and passed through a subaseal at the top of the electrical conduit, and was connected by means of soft PVC tubing to a three-way stopcock from which gas samples were taken. The volume of the sampling tube was less than 1ml. Either air or water was sampled depending on the height of the water table, since the porous cup was permeable to both.

The porous cup was tapered from the flange so that, when it was inserted into an augered hole, soil could not smear and seal the surface. After installation the hole was backfilled with tightly packed soil to prevent diffusion of air from the soil surface.

To sample soil gases a 5ml syringe fitted with a three-way stopcock was attached to the three-way stopcock of the probe, and after flushing with the first 5ml from the probe the syringe was refilled. An experiment where successive 2ml samples were taken from gas probes (Fig. A.2) showed that the second 5ml sample was representative.

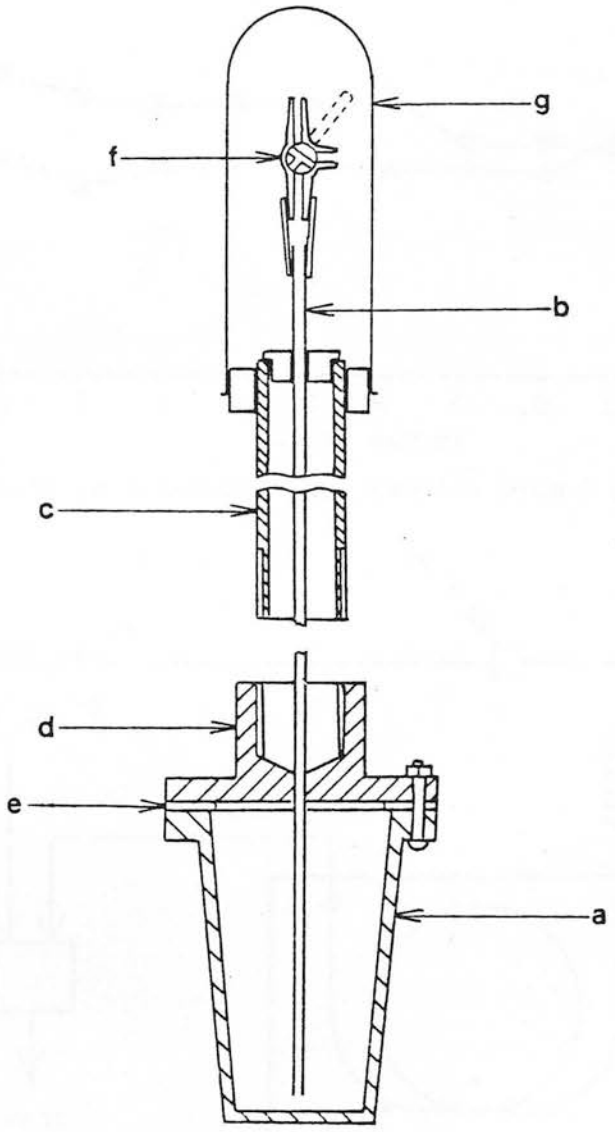


Fig. A.1. Sectional view showing probe construction. a - porous bronze cup, 66mm long, 49mm diameter tapering to 32mm, wall thickness 2.5mm, pore size 5μm; b - stainless steel sampling tube 0.75mm i.d.; c - outer tube (electrical conduit) 19mm o.d. with external thread; d - flange (with internal thread) through which sampling tube is soldered, e - neoprene gasket; f - nylon 3-way stopcock attached to sampling tube by soft PVC sleeve; g - plastic cover, removed while sampling

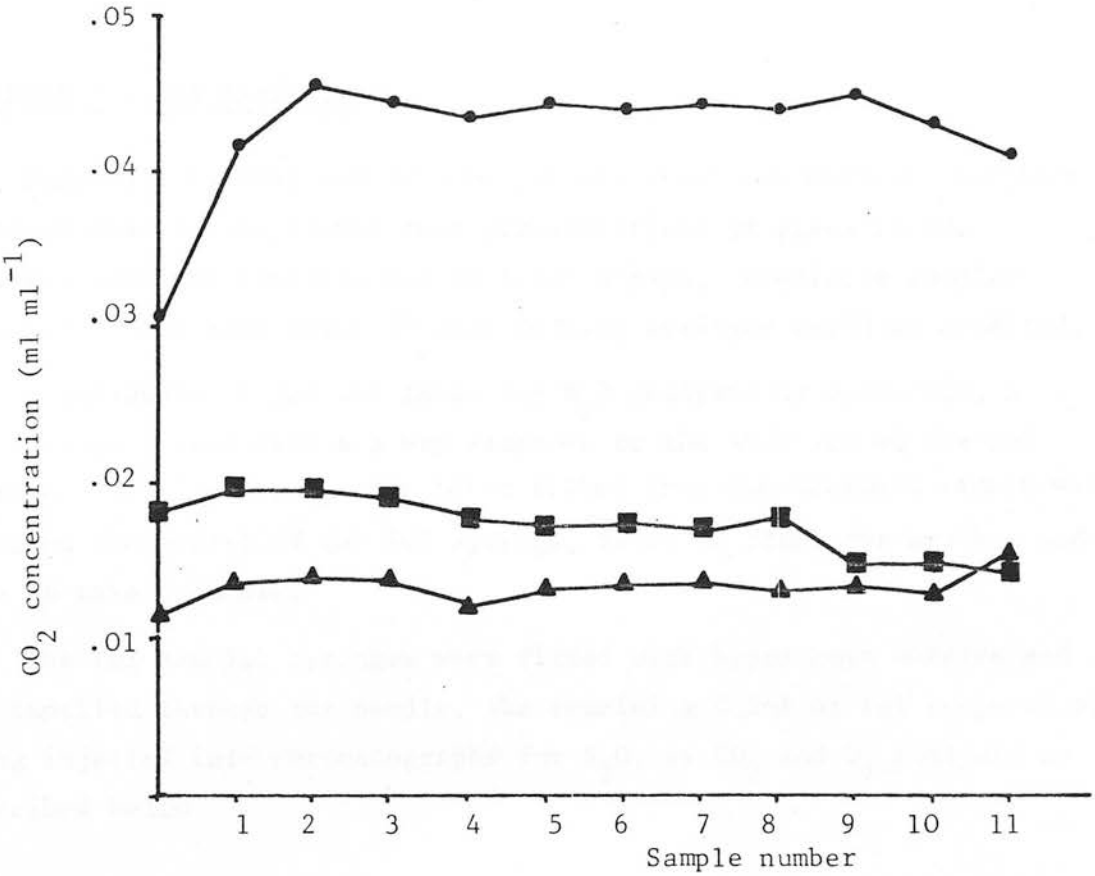


Fig. A.2. CO_2 concentrations in successive 2ml samples from 3 probes

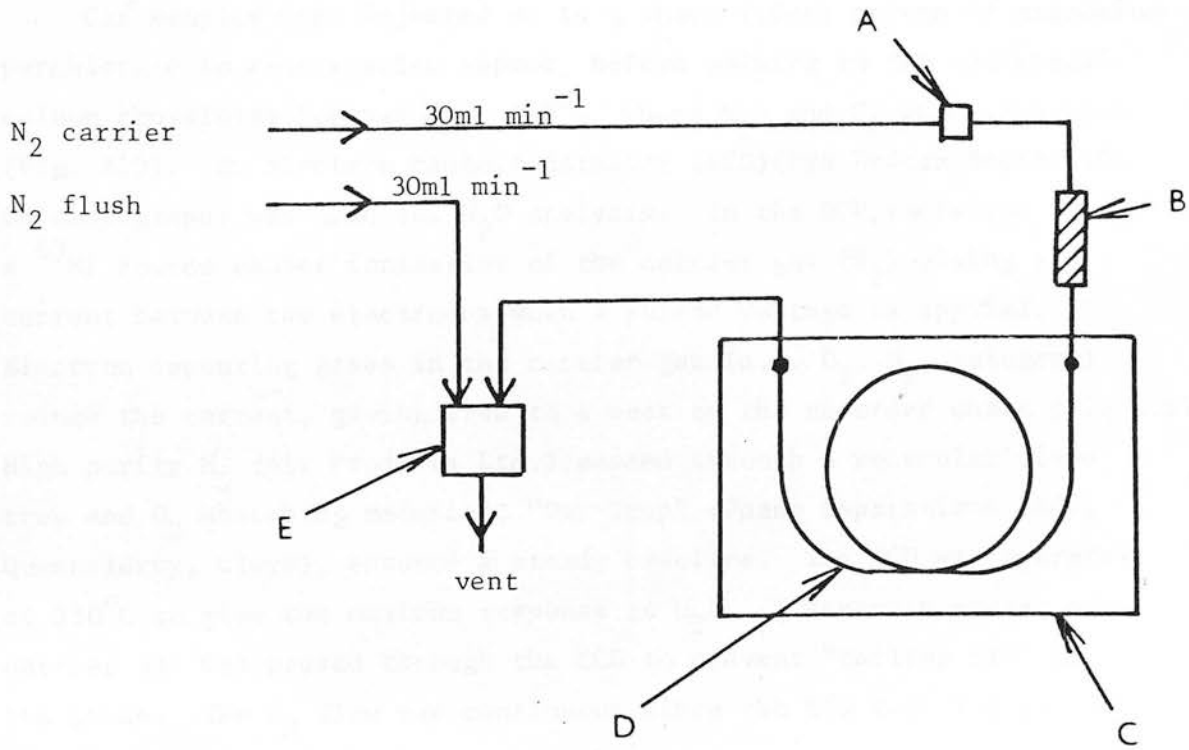


Fig. A.3. N_2O detection using an electron capture detector.
A - injection port; B - glass drying column containing magnesium perchlorate; C - oven at 110°C; D - porapak-Q column 2m long, i.d. 6mm; E - ECD at 350°C

APPENDIX 2 : GAS ANALYSIS

Samples were analysed within 24h of collection whenever possible, although experiments showed that concentrations of gases in the syringes remained constant for at least 3 days. Duplicate samples were taken from each probe so that leaking syringes could be detected.

A subsample of gas was taken for N_2O analysis by connecting a 1ml syringe fitted with a 3 way stopcock to the side arm of the 5ml syringe. The 1ml syringe was twice filled from the original sample while depressing the barrel of the 5ml syringe, first to flush the syringe and then to take a sample.

The 1ml and 5ml syringes were fitted with hypodermic needles and gas expelled through the needle, the remaining 0.5ml or 1ml respectively being injected into chromatographs for N_2O, or CO_2 and O_2 analysis as described below.

N_2O analysis

Gas samples were injected on to a short (10cm) column of magnesium perchlorate to remove water vapour, before passing to the analytical column containing Porapak Q at 110°C, where N_2O and O_2 were separated (Fig. A.3). An electron capture detector (ECD)(Pye Unicam Series 104 Chromatograph) was used for N_2O analysis. In the ECD, radiation from a ^{63}Ni source causes ionisation of the carrier gas (N_2) giving a current between two electrodes when a pulsed voltage is applied. Electron capturing gases in the carrier gas (e.g. O_2, N_2, halogens) reduce the current, giving rise to a peak on the recorder chart (Fig.A.8). High purity N_2 (Air Products Ltd.) passed through a molecular sieve trap and O_2 absorbing material, "Oxy-Trap" (Phase Separations Ltd., Queensferry, Clwyd), ensured a steady baseline. The ECD was operated at 350°C to give the maximum response to N_2O. A separate stream of carrier gas was passed through the ECD to prevent "tailing off" of the peaks. The N_2 flow was continuous since the ECD took 2 days to reach maximum sensitivity.

At a pulse interval of 150μs and 500μs the linear range was 0-4 x 10^{-5} ml ml^{-1} and 0-4 x 10^{-6} ml ml^{-1} respectively (Figs. A.4 and A.5),

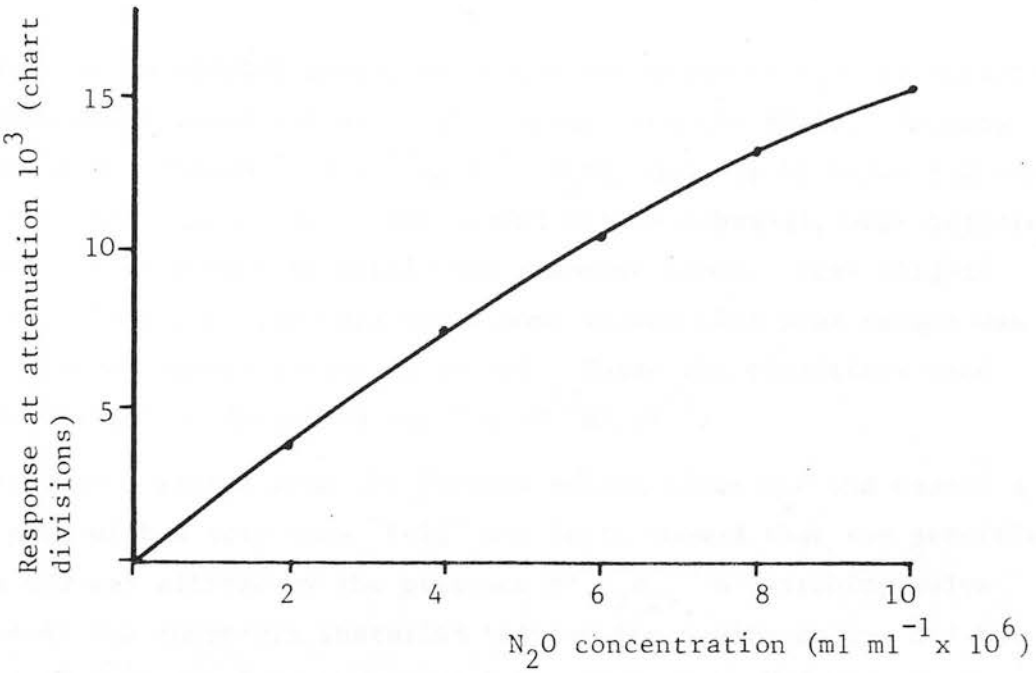


Fig. A.4. Response curve at 500μs

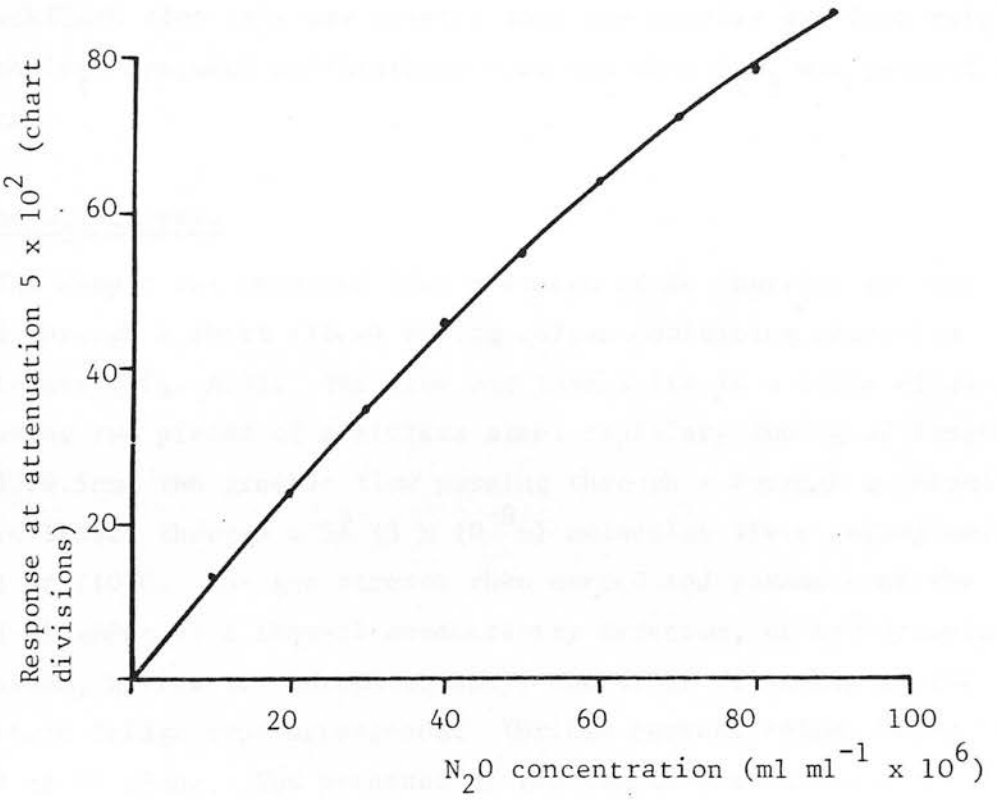


Fig. A.5. Response curve at 150μs

therefore since samples contained a wide variation in N_2O concentration the 150μs pulse space was used for samples from the field. Varying amounts of a standard (1×10^{-4} ml ml^{-1} N_2O), made up by injecting pure N_2O into a flask of known volume sealed with a subaseal, were injected into the chromatograph to obtain the response curve. Peak heights were used since a preliminary experiment showed that peak height was independent of sample volume up to 1ml. Under the conditions used the lower limit of detection was 2×10^{-7} ml ml^{-1}.

Acetylene elutes from the Porapak column after N_2O and caused a large peak with a very long "tail" and tests showed that the sensitivity of the ECD was altered by the presence of C_2H_2. A switching valve (Fig. A.6) was therefore installed between the drying column and the oven so that in samples containing C_2H_2, as soon as N_2O eluted, the flow of N_2 through the column could be reversed, i.e. the C_2H_2 flushed out. Meanwhile a second Porapak column was used for the next sample. The backflush flow rate was greater than the carrier gas flow rate. Nevertheless frequent calibrations were run when C_2H_2 was present in samples.

CO_2 and O_2 analysis

The sample was injected into a stream of He carrier gas and passed through a short (10cm) drying column containing magnesium perchlorate (Fig. A.7). The flow was then split in a ratio of about 32:1 using two pieces of stainless steel capillary tubing of length 27 and 19.5cm, the greater flow passing through a Porapak Q column and the lesser through a 5Å (5×10^{-8}m) molecular sieve column maintained at 110°C. The gas streams then merged and passed over the heated filament of a thermal conductivity detector, or katharometer Pye Unicam, Series 104 Chromatograph), the other filaments in the Wheatstone Bridge type arrangement (bridge current 165mA) being in a flow of He alone. The presence of the sample gas causes a change in temperature and therefore resistance of the filaments causing a signal resulting from an imbalance in the bridge. The thermal conductivity of He (over 10x that of CO_2, O_2 and N_2) makes it suitable as a carrier gas. Only 30 minutes were required for stable operating

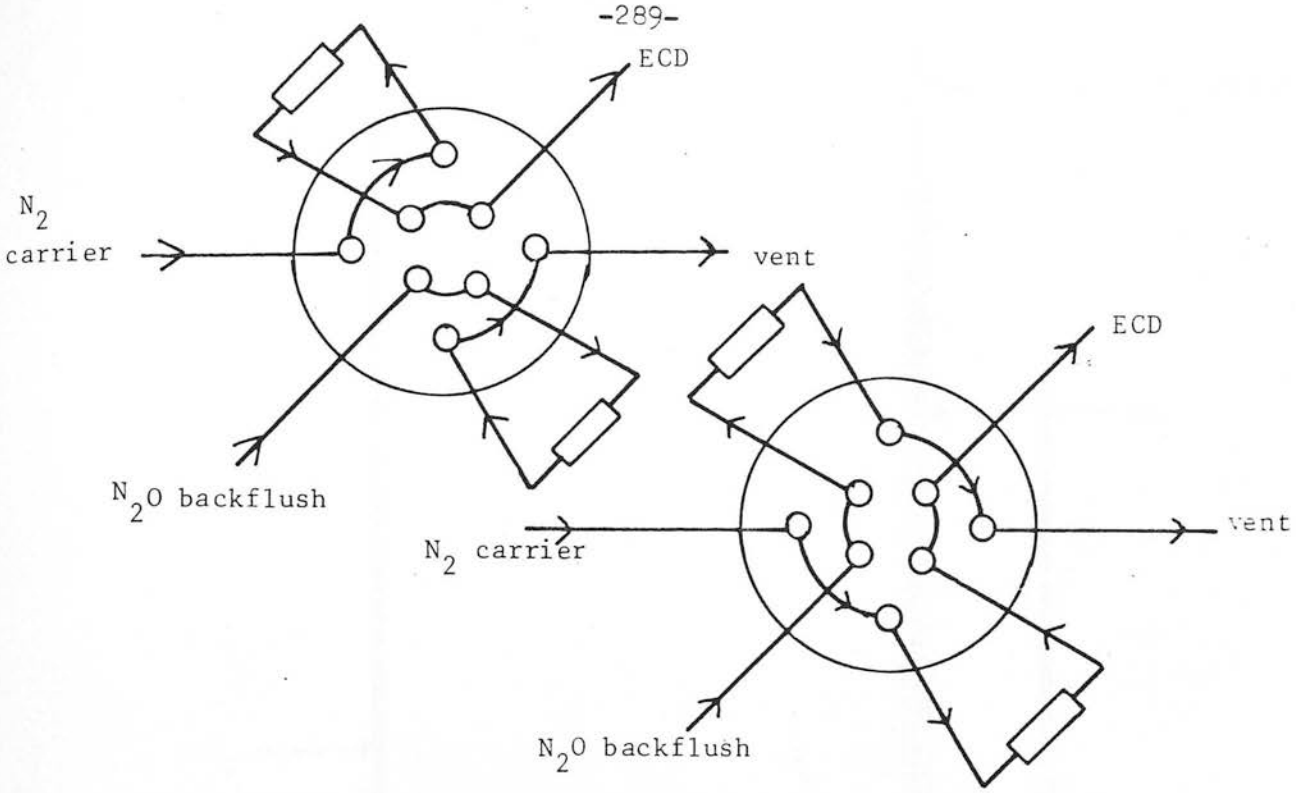


Fig. A.6. 8 way switching valve used when samples for N_2O analysis contained C_2H_2 showing the two positions

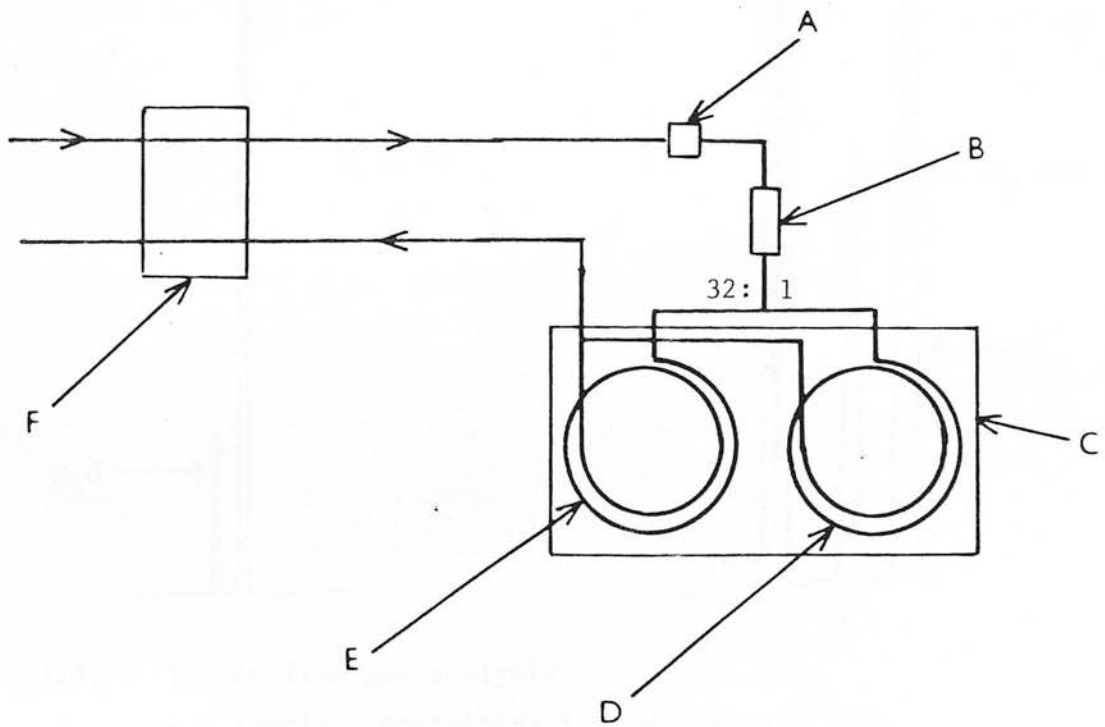


Fig. A.7. Katharometer detector for analysis of O_2 and CO_2. A - injection port; B - glass drying column containing magnesium perchlorate; C - oven at 110°C; D - molecular sieve column 1.5m long, 3mm i.d.; E - porapak Q column 1.5m long, 6mm i.d.; F - katharometer detector

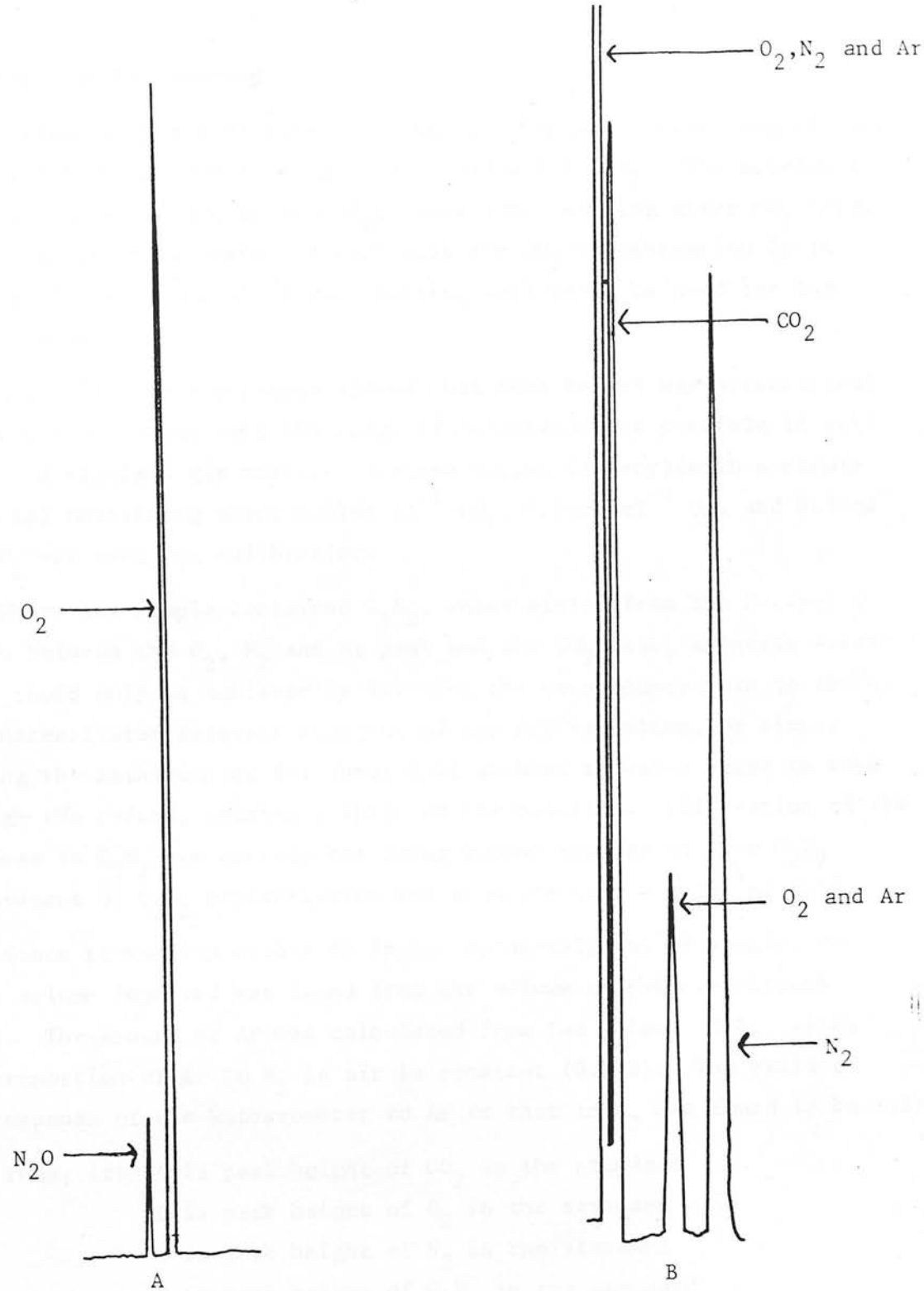


Fig. A.8. Traces from gas analysis

A - sample containing 1×10^{-6} ml ml^{-1} N_2O

B - sample containing 0.01ml CO_2, 0.20ml O_2 and 0.79ml N_2

conditions to be achieved.

Nitrogen, O_2 and Ar passed through the Porapak without significant retention giving a single large peak, followed by CO_2. The molecular sieve separated O_2 and Ar from N_2, these gases eluting after CO_2 (Fig. A.8). The split of gases was such that for CO_2 concentration up to 0.025ml ml^{-1} the same attenuator setting (x2) could be used for the whole sample.

A preliminary experiment showed that peak height was proportional to gas concentration over the range of concentrations possible in soil gases. A standard gas mixture (British Oxygen Company)(with accurate analysis) containing about 0.01ml ml^{-1} CO_2, 0.20ml ml^{-1} O_2, and 0.79ml ml^{-1} N_2 was used for calibration.

Where the sample contained C_2H_2, which eluted from the Porapak Q column between the O_2, N_2 and Ar peak and the CO_2 peak, adequate separation could only be achieved by lowering the oven temperature to 100°C. This necessitated frequent changing of the drying column, or simply leaving the katharometer for about half an hour if water began to come through the column, causing a shift in the baseline. Calibration of the response to C_2H_2 was carried out using 0.05ml samples of pure C_2H_2. Measurement of C_2H_2 concentration was accurate to $\pm 4 \times 10^{-4}$ml ml^{-1}.

Since it was impossible to inject accurately 1ml of sample, the total volume injected was found from the volume of the constituent gases. The amount of Ar was calculated from the volume of N_2, since the proportion of Ar to N_2 in air is constant (0.012). The ratio of the response of the katharometer to Ar to that to N_2 was found to be 1.233.

Thus, if: A is peak height of CO_2 in the standard
B is peak height of O_2 in the standard
C is peak height of N_2 in the standard
D is peak height of C_2H_2 in the standard

a is the volume of CO_2 in the standard (ml)
b is the volume of O_2 in the standard (ml)
c is the volume of N_2 in the standard (ml)
d is the volume of C_2H_2 in the standard (ml)

w is the peak height of CO_2 in the sample
x is the peak height of O_2 in the sample
y is the peak height of N_2 in the sample
z is the peak height of C_2H_2 in the sample

Then: Volume of CO_2 in the sample = wa/A (ml) A.1

Volume of N_2 in the sample = yc/C (ml) A.2

Volume of O_2 in the sample = (x - (1.233 X 0.012 y))b/B (ml) A.3

Volume of Ar in the sample = 0.012yc/C (ml) A.4

Volume of C_2H_2 in the sample = zd/D (ml) A.5

Total Volume = wa(A + (x - 0.0148y)b/B + 1.012yc/C + zd/D (ml) A.6

Concentrations were calculated using a programmable calculator. The method gave an accuracy for O_2 and N_2 of $\pm$ 2 x 10^{-3} ml ml^{-1}, for CO_2 of $\pm$ 8 x 10^{-5} ml ml^{-1}, and for C_2H_2 of $\pm$ 2 x 10^{-4} ml ml^{-1}.

APPENDIX 3: ANALYSIS OF WATER SAMPLES AND SAMPLES FROM INCUBATION VESSELS

A. Water Samples

Syringes containing water were stored in the refrigerator prior to analysis. Dissolved O_2 was measured directly while N_2O, C_2H_2 and CO_2 were determined by equilibrating the sample with air in a closed container and measuring gas concentrations in the headspace, since they have relatively high solubilities.

Dissolved O_2

After being allowed to come to equilibrium, and recording the amounts of air and water, the syringe was held upright in a clampstand and 20µl of water removed, using a microsyringe. The water sample was injected onto glass beads in a copper tube at 160°C (see Fig. A.9). The water vapour was retained on a 10cm column of Porapak T while the gases passed through and into the analytical column (Porapak Q) and then to the ECD. When the peak due to O_2 appeared, a valve was switched to backflush the Porapak T column and substitute a similar second column for the next sample. A sample of water in equilibrium with lab air was used as a standard and the O_2 concentration in the sample expressed as an equivalent concentration in air in equilibrium with the water sample.

Dissolved N_2O, C_2H_2 and CO_2

Following O_2 analysis, the syringe was fitted with a hypodermic needle, all the air expelled and the water injected into a weighed 'vacutainer' (B.D.H. Ltd.) of volume 5.53ml which was reweighed to determine the volume of water and shaken mechanically for 10 minutes so that dissolved N_2O, CO_2 and C_2H_2 equilibrated with the air in the vacutainer. Two 0.5ml samples of air were removed for CO_2 and C_2H_2, and N_2O analysis respectively (see Appendix 2).

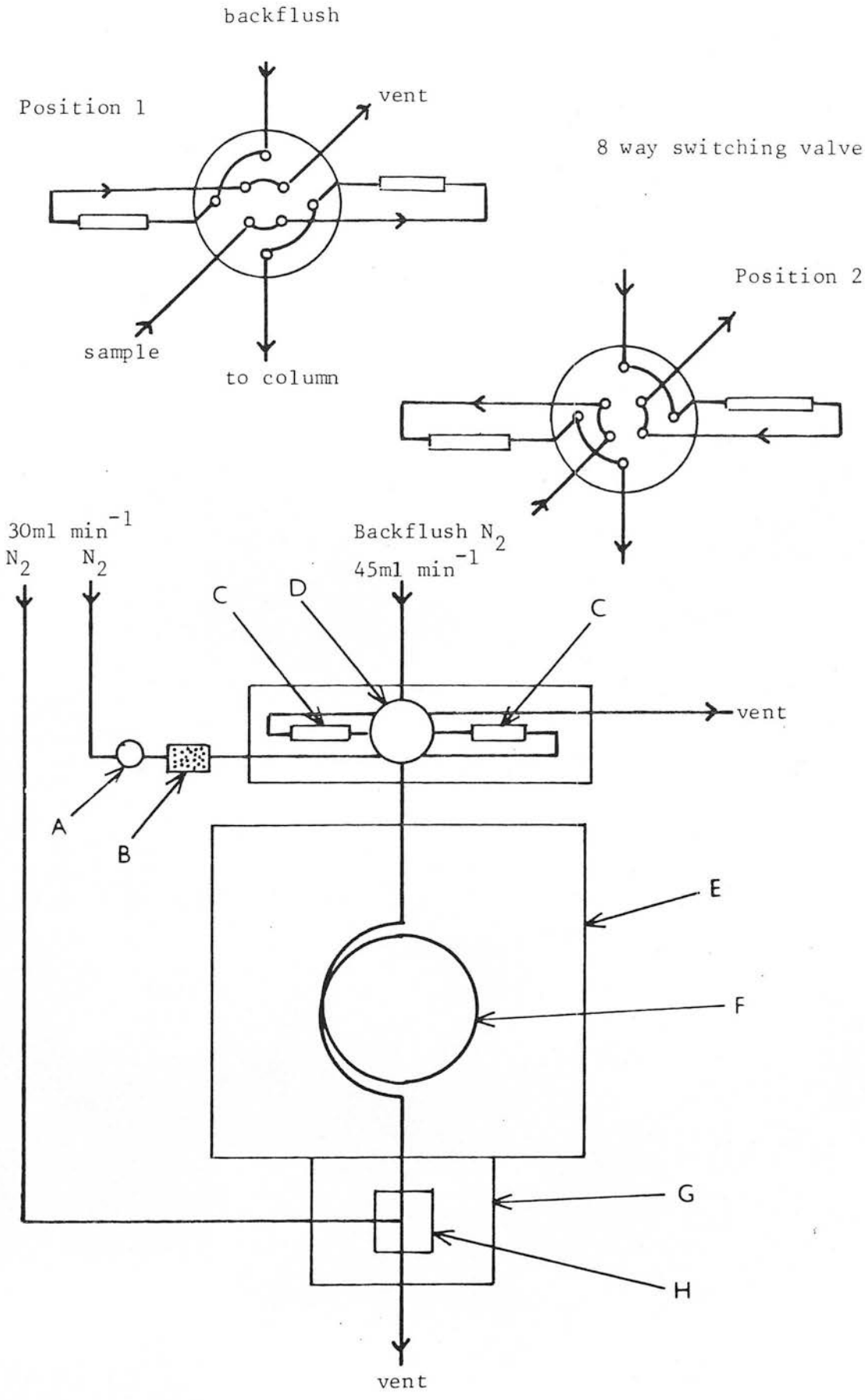


Fig. A.9. ECD arrangement for measurement of dissolved O_2 in water samples. A - injection port; B - glass bead column at 160°C; C - Porapak-T column; D - 8 way switching valve; E - column oven at 110°C; F - Porapak-Q column 1.5m long, i.d. 6mm; G - ECD oven at 130°C; H - ECD

Calculations

Concentrations in the gas phase which would be in equilibrium with the water samples were calculated so that results for water samples and gas samples were comparable. When syringes are brought from the field to the laboratory, a redistribution of dissolved gas in the liquid and gaseous phases takes place, because of the effect of the temperature change on solubility.

a) Oxygen

Let sw = volume water in the syringe (ml

sa = volume air in the syringe (ml)

T_1 = temperature in the laboratory (K)

T_2 = temperature in the field (K)

α_1, α_2 = absorption coefficient of the gas at T_1, T_2 (ml of gas, corrected to STP which dissolves in 1ml of water under an atmosphere of the gas)

α_1^1, α_2^1 = corrected absorption coefficient (ml of the gas at T_1 or T_2 which dissolves in 1ml water under an atmosphere of the gas = $\alpha T/273$)

x_1 = concentration of O_2 in equilibrium with the water sample as used for analysis (ml ml^{-1})

x_2 = concentration of O_2 in equilibrium with water in syringe as taken in the field.

(Absorption coefficients were taken from "The Handbook of Chemistry and Physics (37th edition, 1955-56, P.1606-1609) those for N_2O and CO_2 being calculated from Henry's Law Constant).

Volume of O_2 in the syringe = vol. O_2 in air + vol. O_2 in water

At T_1, in the laboratory, volume $O_2 = x_1(sa + sw\alpha_1^1)$ (ml) A.7

At T_2, in the field, volume $O_2 = x_2(sa + sw\alpha_2^1)$ (ml) A.8

$\therefore$ at T_1, in the laboratory, volume $O_2 = x_2(sa + sw\alpha_2^1)T_1/T_2$ (ml) A.9

Since A.7 and A.9 are identical:

$$x_2 = x_1(sa + sw\alpha_1^1)T_2 / (sa + sw\alpha_2^1)T_1 \quad (ml\ ml^{-1}) \qquad A.10$$

Hence x_2 could be calculated since all other variables were known.

b) CO_2, N_2O or C_2H_2

Let V_a = volume of air in the vacutainer (ml)
V_w = volume of water in the vacutainer (ml)
x_1 = the concentration of the gas measured in the vacutainer (ml ml^{-1})
x_2 = the concentration of the gas in equilibrium with water in the syringe in the field at T_2 (ml ml^{-1})

Then volume of the gas in the vacutainer at T_1 =

$$x_1(V_a + \alpha_1^{1}V_w) \quad (ml)$$

Concentration of the gas in the syringe water at T_1 =

$$x_1(V_a + \alpha_1^{1}V_w)/V_w \quad (ml)$$

Concentration of the gas in the syringe air in equilibrium with the water at T_1 = $x_1(V_a + \alpha_1^{1}V_w)/V_w\alpha_1^{1}$ (ml ml^{-1})

Total gas in the syringe =

$$x_1(V_a + \alpha_1^{1}V_w)(S_a + \alpha_1^{1}S_w)/P_w\alpha_1^{1} \quad (ml) \qquad A.11$$

But total gas in the syringe at T_1 =

$$x_2(S_a + S_w\alpha_2^{1})T_1/T_2 \quad (ml) \qquad A.12$$

Therefore $x_2 = x_1(V_a + \alpha_1^{1}V_w)(S_a + \alpha_1^{1}S_w)T_2/P_w\alpha_1^{1}(S_a + \alpha_2^{1}S_w)T_1$ A.13

Hence x_2 could be calculated since all other variables were known.

B. Samples from Incubation Vessels

The gas concentration in the headspace of the vessel (x ml ml^{-1}) was measured as described in Appendix 2.

Let: V = volume of vessel (ml)
V_s = w/2.66 = volume occupied by w g of soil (particle density 2.66 g ml^{-1}) (ml)

V_w = volume of water added (ml)

T = temperature during the incubation (^oK)

α^1 = corrected absorption coefficient (as defined in Appendix 3.A) at T ($ml\ ml^{-1}$)

Then: Volume of gas in the headspace of the vessel

$$= (V - V_s - V_w)x \text{ ml}$$

Volume of gas in the aqueous phase = $V_w\alpha^1 x$ ml

Total volume of gas at $0^oC = (V - V_s - V_w + V_w\alpha^1)273x/T$ ml (A.9)

If during the incubation, the concentration of the gas is increasing, the measured concentration at the nth sampling was corrected for the quantity of gas removed in previous samples by amending equation A.9 to:

$$\text{Corrected total volume of gas at } 0^oC = ((V - V_s - V_w + V_w\alpha^1)x + \sum_{i=1}^{i=n-1} v_i x_i)273/T \text{ (ml)} \quad (A.10)$$

where v_i is the volume removed from the vessel on the ith sampling occasion

x_i is the measured concentration of the gas on the ith sampling occasion.

APPENDIX 4: SLURRY AND FERTILISER APPLICATION

Slurry is a suspension of faecal matter and some straw in water and urine varying in composition depending on the age of the animals, their feed, and the water content. The solid material tends to settle out in the storage tanks. Cow slurry from the collection tank at Langhill Farm was analysed for total N prior to each application to determine the volume required. Slurry in the tank was stirred mechanically and pumped out into a metal tank for transport to the field. At the experimental site the slurry was stirred continually by hand, measured out into calibrated buckets and transferred to watering cans for application to the plots, which were subdivided with string into $1m^2$ sections to facilitate uniform application of the slurry. During this procedure slurry samples were taken to determine accurately N added.

Since the farm later acquired a separating plant, only liquid slurry was applied to the microplots. A sufficient volume was collected for the application, and following analysis, the required volume was added.

For application to the field plots, the inorganic fertiliser (calcium nitrate) was weighed out in quantities sufficient for $1m^2$. The plots were marked out with string into $1m^2$ sections and the fertiliser was broadcast by hand in each section as evenly as possible. For each microplot sufficient calcium nitrate was weighed out and scattered as evenly as possible over the soil within the area confined by the PVC pipe.

APPENDIX 5: ANALYSIS OF SLURRY AND HERBAGE

Slurry

Since analysis showed that the solid particles of slurry remaining on a 2mm sieve contained less than 5% of the total N, only the portion of slurry passing through the sieve was analysed routinely. The liquid portion was stirred well in a bucket and a 500ml sample taken in a beaker and stirred using a magnetic stirrer. A 1ml sub-sample was taken from the beaker using an automatic pipette with a cut-off end. Sampling from the bucket was repeated 4 times and 2 samples were taken from each beaker. The 8 sub-samples were analysed by Kjeldahl digestion as described below.

Herbage

Grass in the field plot was cut with an Allen Scythe or a Flymo mower. Grass from each plot was weighed in the field using a spring balance and a sample was taken, dried in an oven at 60°C, milled and analysed by Kjeldahl digestion as described below.

Digestion

A micro-Kjeldahl method, using selenium as a catalyst, was used enabling analysis of N, P and K to be carried out on the same digest (Chapman and Pratt, 1961). Samples of slurry (1ml) or herbage (0.25g) were placed in a Kjeldahl flask and 3ml of 60 volume H_2O_2 added, followed by 4ml of a solution containing 0.35g selenium in 1 litre of concentrated H_2SO_4. This was heated until the digest cleared and then for a further hour, allowed to cool, and made up to 50ml with water in a volumetric flask. At least two blank samples were included with each set of samples.

Nitrogen Analysis

From the diluted digest 10ml were transferred to a Markham still and about 10ml of a 40% solution of NaOH added. Steam was then passed through and the condensed distillate containing ammonia passed

into 2% boric acid containing bromocresol green and methyl red indicator (Bremner, 1965). The ammonia was then titrated with 0.005M H_2SO_4.

Phosphate Analysis

Analysis was by a colorimetric method using vanadate (Kitson and Mellon, 1944) described by Jackson (1958). A working solution was made up from 150ml of stock solution (40g ammonium molybdate, 2g ammonium vanadate and 150ml concentrated nitric acid made up to 1 litre with distilled water) and 450ml of distilled water. Using a 25ml flask, 20ml of the working solution were added to a 5ml aliquot of the diluted digest, and the yellow colour developed in a water bath at 37°C for at least 15 minutes before reading at 395nm in a 1cm cell in a spectrophotometer. A standard curve was prepared by using 5ml of distilled water or of solutions of di-sodium hydrogen phosphate (Na_2HPO_4) in the concentration range 2-8µg P ml^{-1}.

Potassium Analysis

The diluted digests were analysed for potassium using a flame photometer and measured against a standard curve obtained with distilled water and solutions of potassium chloride of concentration 10-100µg K ml^{-1}.

APPENDIX 6: ANALYSIS OF SOIL CORES FOR NITRATE AND AMMONIUM

Collection of Cores

Soil cores were taken at least 0.5m away from a probe, from the edge of a plot, or from where a core had previously been taken, by driving a corer into the ground to at least 40cm. (Soil from below the 40cm depth is referred to as from the 40+ depth in the text). The hole was filled with bentonite to ensure the aeration of the surrounding area was not affected by loosely packed soil. Each core was cut into 10cm portions, grass shoots were removed from the 0-10cm portion, and the soil was sieved field moist through a 2mm sieve and stored in a cold room at $4^{o}C$. A preliminary study with a variety of soils showed that at $4^{o}C$ extractable NH_4^+ and NO_3^- remained almost constant for at least 8 weeks whereas at higher temperatures NO_3^- concentrations tended to increase with time and NH_4^+ to fluctuate widely, even at a moisture content half that of field capacity.

The soils were extracted in 2M potassium chloride (KCl) in the ratio 5g soil to 25ml KCl for one hour on a shaker using plastic centrifuge tubes, filtered through No. 42 filter paper and stored in glass bottles. The moisture content of the soil was determined by drying in an oven at $105^{o}C$.

Ammonium Analysis - Method 1

Ammonium was determined by a colorimetric method involving the formation of an ammonia-phenol complex in the presence of sodium nitroprusside as a catalyst and hypochlorite as an oxidising agent (Kempers, 1974). Steam distillation was necessary since metal ions in the extract interfered with the colour formation. Duplicate aliquots of the KCl extract were steam distilled with 50mg of magnesium oxide using apparatus similar to Bremner's (1965a), until about 15ml of the condensed distillate containing ammonium had passed into a 25ml volumetric flask containing 2ml of a phenol-nitroprusside reagent. After mixing well, 4ml of buffered hypochlorite solution was added and the flask made up to volume. After 1 hour the absorbance at 636nm

was measured using a 1cm cell. Duplicate blank solutions of 2M KCl were also distilled. A standard curve was obtained using distilled water or ammonium chloride solutions containing 2-15µg ml^{-1} NH_4^+. Distilled deionised water was used both for the extracting solution and for all reagents used in ammonium analysis to overcome contamination problems.

Ammonium Analysis - Method 2

Ammonium in the KCl extract was determined by continuous-flow analysis (Fig. A.10). The method is based on that used by Crooke and Simpson (1971), but modified to allow for the much lower concentrations in KCl extracts compared with in Kjeldahl digests. An emerald green colour is formed between NH_4^+ and a weakly alkaline mixture of sodium salicylate and dichloroisocynurate in the presence of sodium nitroprusside as a catalyst, which makes the reaction very sensitive. The colour is measured after passing through a delay coil in a water bath at 37°C for 15min and the colour read at 660nm in a 1cm cell. If necessary, extracts were diluted with 2M KCl. Ammonium chloride solutions of 0.5 - 2.0µg NH_4^+-N ml^{-1} in 2N KCl were used as standards. Sampling time was 1min with 30s wash time between samples.

Analysis of Nitrate

Nitrate in the KCl extract was determined colorimetrically by continuous-flow analysis (see Fig. A.11) using a method described by Henrikson and Selmer-Olsen (1970). Nitrate was first reduced to NO_2^- in a copperised-cadmium column, formed by washing cadmium in a solution of hydrochloric acid followed by copper sulphate, which could be regenerated whenever the intensity of colour decreased. The NO_2^- underwent a diazotisation reaction with sulphanilamide, and the product coupled with N-1-naphthylethylenediamine dihydrochloride to form an intense magenta coloured azo-dye, the absorbance of which was measured at 520nm in a 1cm cell. A calibration curve was obtained from 2M KCl and solutions containing 0.5 - 2.0µg N ml^{-1} as potassium nitrate in 2M KCl. Extracts were diluted with 2M KCl

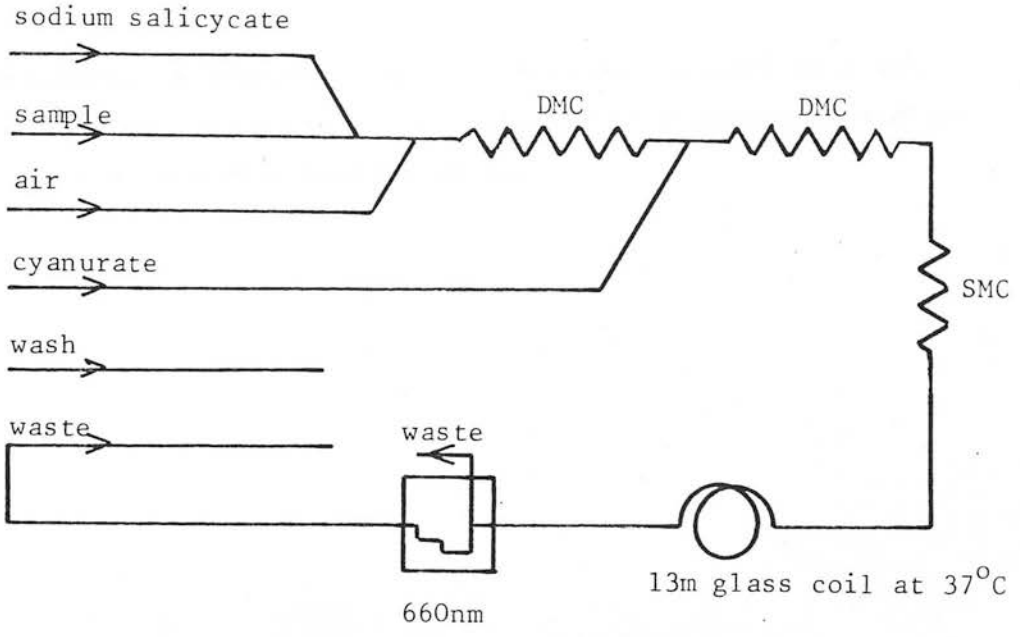


Fig. A.10. Ammonium analysis by continuous flow method. DMC - double mixing coil. SMC - single mixing coil.

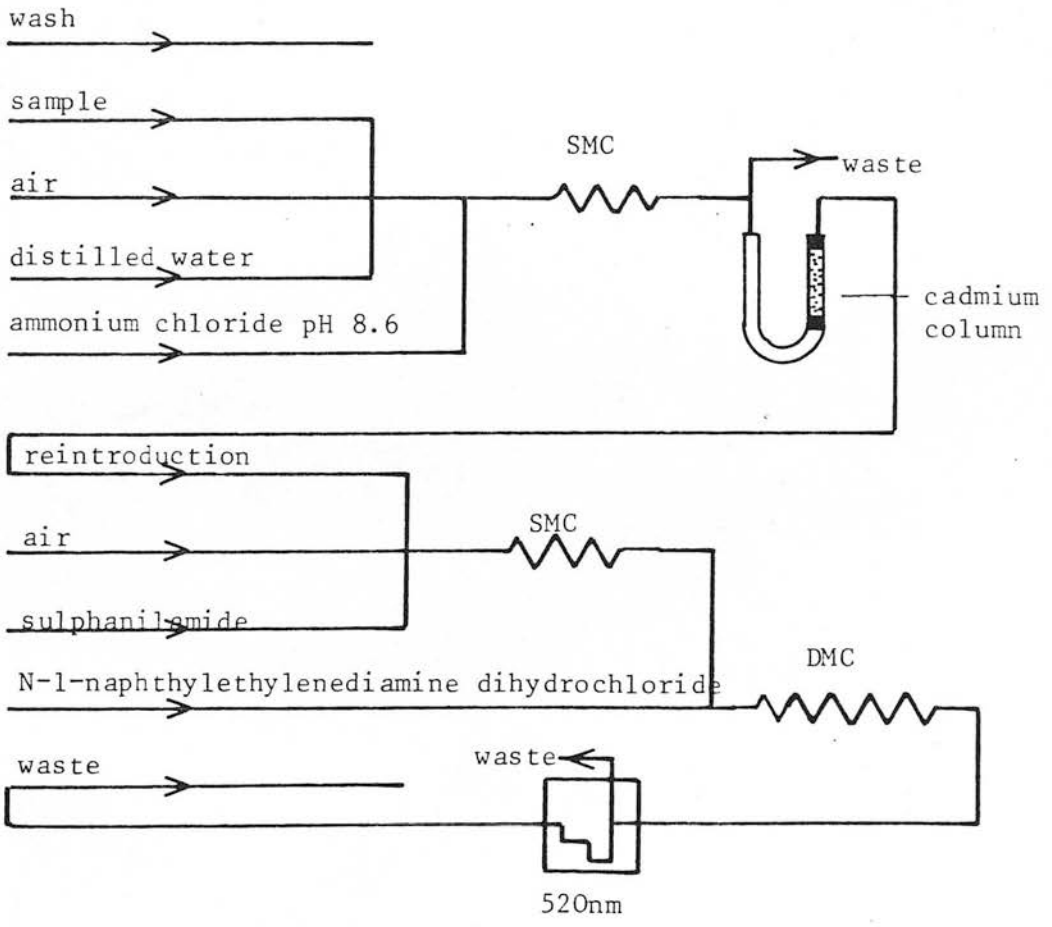


Fig. A.11. Determination of nitrate by continuous flow analysis. SMC - single mixing coil; DMC - double mixing coil

where necessary. A sampling time of 2 minutes followed by a wash time of 3 minutes was necessary for full colour development and to prevent interference from other samples.

APPENDIX 7: DATA FOR CHAPTER 2

A. Treatment Means For N_2O, O_2 and CO_2.

Date	Treatment Means at the 15cm depth.								
	Nitrous Oxide			Oxygen			Carbon Dioxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x 10^6			ml ml^{-1}			ml ml^{-1} x 10^2		
5.9.78	3.0	8.8	10.4	0.161	0.084	0.137	3.1	6.9	4.2
8.9.78	4.0	32.5	28.8	0.159	0.096	0.149	3.7	6.2	4.9
12.9.78	1.2	26.3	33.8	0.181	0.149	0.180	2.3	6.6	3.0
19.9.78	1.1	6.3	7.5	0.179	0.181	0.192	1.6	3.4	2.2
25.9.78	1.0	2.8	2.4	0.191	0.192	0.192	1.8	2.0	1.5
2.10.78	2.2	4.5	9.5	0.165	0.137	0.150	2.1	4.1	3.7
9.10.78	1.3	3.7	3.0	0.169	0.174	0.191	2.7	4.1	2.1
16.10.78	0.8	2.2	2.6	0.179	0.168	0.188	2.2	3.4	2.1
23.10.78	0.3	0.6	1.0	0.189	0.188	0.199	1.5	2.1	1.4
30.10.78	0.8	1.5	0.8	0.189	0.189	0.202	1.8	2.1	0.8
6.11.78	2.7	3.5	4.4	0.168	0.162	0.167	2.1	3.3	2.8
20.11.78	2.0	2.2	3.6	0.129	0.156	0.164	2.2	2.6	2.0
27.11.78	1.5	1.8	3.3	0.165	0.180	0.182	1.8	2.0	1.6
3.12.78	1.4	0.9	1.1	0.171	0.184	0.187	1.7	1.9	1.8
11.12.78	2.1	3.0	6.2	0.159	0.170	0.176	1.6	2.1	1.6
18.12.78	1.5	1.5	1.0	0.163	0.171	0.184	1.8	1.6	1.5
3.1.79	2.8	3.1	3.1	0.149	0.186	0.185	1.7	1.4	1.3
8.1.79	3.6	4.1	2.3	0.147	0.183	0.191	1.6	1.3	1.0
15.1.79	7.0	5.6	3.8	0.167	0.188	0.195	1.3	1.4	0.8
22.1.79	8.8	3.7	2.0	0.158	0.186	0.187	0.9	0.9	0.6
29.1.79 - 21.2.79	NO DATA								
28.2.79	130.3	94.6	225.9	0.107	0.184	0.157	0.8	0.7	0.9
7.3.79	25.8	6.6	48.4	0.173	0.196	0.186	1.0	0.3	0.6
15.3.79	8.7	5.9	21.3	0.176	0.198	0.189	1.0	0.5	0.5
23.3.79	5.6	6.7	18.0	0.159	0.190	0.189	0.5	0.4	0.3

CONTINUED/

Treatment Means at the 15cm depth (CONTINUED)

Date	Nitrous Oxide			Oxygen			Carbon Dioxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x 10^{6}			ml ml^{-1}			ml ml^{-1} x 10^{2}		
28.3.79	3.6	3.9	8.8	0.186	0.188	0.190	0.7	0.3	0.2
6.4.79	1.7	3.2	1.9	0.188	0.193	0.192	0.5	0.5	0.4
12.4.79	1.2	3.7	3.9	0.178	0.188	0.188	0.7	0.6	0.4
19.4.79	2.1	2.0	6.4	0.167	0.186	0.175	1.9	1.3	1.6
25.4.79	1.9	2.6	4.1	0.178	0.189	0.178	1.6	1.2	1.8
1.5.79	1.2	1.2	3.1	0.180	0.193	0.184	1.5	1.1	1.4
9.5.79	1.0	1.1	2.3	0.176	0.186	0.191	2.2	1.8	1.5
16.5.79	0.7	0.6	1.7	0.180	0.186	0.187	3.2	2.2	2.6
23.5.79	1.1	0.9	1.9	0.147	0.164	0.164	4.5	3.3	4.0
29.5.79	1.8	1.8	1.8	0.107	0.127	0.111	6.1	5.6	5.8
7.6.79	1.8	2.6	0.5	0.178	0.193	0.186	4.0	3.0	3.4
13.6.79	0.9	1.3	0.7	0.177	0.186	0.189	3.6	2.8	2.9
28.6.79	0.5	0.7	0.5	0.200	0.202	0.202	1.2	1.1	1.1
5.7.79	0.4	0.4	0.5	0.207	0.215	0.208	0.9	0.6	0.8
12.7.79	0.4	0.5	0.6	0.205	0.206	0.207	0.5	0.7	0.5

N.B. All means are calculated from transformed data and the reverse transform of the mean is presented above.

Date	Treatment Means at the 30cm Depth								
	Nitrous Oxide			Oxygen			Carbon Dioxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml $ml^{-1} \times 10^{6}$			ml ml^{-1}			ml $ml^{-1} \times 10^{2}$		
5.9.78	2.4	5.5	2.5	0.151	0.087	0.166	4.3	6.9	3.0
8.9.78	4.5	13.1	16.8	0.137	0.059	0.148	4.9	8.2	4.7
12.9.78	4.1	68.0	15.2	0.156	0.116	0.169	4.2	6.3	3.4
19.9.78	2.0	26.0	9.5	0.177	0.155	0.183	3.8	4.4	3.7
25.9.78	1.5	9.2	3.6	0.182	0.176	0.192	3.3	3.2	1.9
2.10.78	5.5	14.0	13.2	0.141	0.134	0.154	4.0	4.1	3.2
9.10.78	1.8	6.5	6.5	0.156	0.160	0.184	3.7	4.6	2.9
16.10.78	1.1	4.5	3.5	0.167	0.166	0.177	3.3	3.8	2.8
23.10.78	0.9	1.1	1.6	0.183	0.182	0.194	3.0	2.7	1.9
30.10.78	1.6	2.1	0.9	0.182	0.185	0.198	3.2	2.5	1.1
6.11.78	3.2	5.3	5.8	0.155	0.158	0.171	3.4	3.4	2.5
20.11.78	4.8	4.4	9.8	0.145	0.121	0.153	3.2	3.2	2.1
27.11.78	2.8	3.0	5.6	0.170	0.156	0.157	3.7	2.6	2.2
3.12.78	1.7	1.7	2.8	0.185	0.171	0.179	2.9	2.3	2.1
11.12.78	1.9	5.4	6.7	0.187	0.161	0.180	1.4	2.0	0.6
18.12.78	1.6	5.1	6.6	0.179	0.170	0.178	2.0	2.4	1.4
3.1.79	4.3	11.0	20.9	0.176	0.140	0.161	2.2	2.4	1.5
8.1.79	2.4	16.6	7.5	0.190	0.151	0.183	1.1	1.8	0.7
15.1.79	3.2	10.7	20.5	0.192	0.167	0.189	1.2	1.8	1.8
22.1.79	4.3	7.8	2.6	0.183	0.170	0.180	1.2	1.2	1.1
29.1.79	5.7	7.9	9.5	0.188	0.170	0.183	1.9	1.6	1.1
6.2.79	10.5	16.3	27.1	0.175	0.171	0.179	1.7	1.4	1.0
14.2.79	3.8	2.6	23.6	0.178	0.178	0.177	0.3	0.3	0.8
21.2.79	26.6	46.1	73.0	0.163	0.154	0.166	0.7	0.4	0.6

CONTINUED/

Treatment Means at the 30cm Depth (CONTINUED)

Date	Nitrous Oxide			Oxygen			Carbon Dioxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x 10^{6}			ml ml^{-1}			ml ml^{-1} x10^{2}		
28.2.79	39.2	120.3	262.4	0.189	0.152	0.159	0.5	0.8	0.7
7.3.79	53.5	62.2	117.9	0.177	0.173	0.130	1.7	1.0	1.4
15.3.79	23.1	22.0	98.5	0.167	0.177	0.128	2.4	1.1	2.2
23.3.79	16.0	23.3	63.4	0.190	0.176	0.129	1.7	0.8	2.1
28.3.79	13.1	32.8	6.6	0.192	0.171	0.128	1.2	0.7	1.8
6.4.79	7.9	10.6	3.1	0.169	0.166	0.177	2.1	1.1	1.5
12.4.79	2.9	6.9	51.9	0.192	0.164	0.169	1.0	0.9	1.7
19.4.79	4.1	7.2	7.2	0.168	0.162	0.142	3.1	2.1	2.8
25.4.79	2.9	3.6	4.5	0.179	0.171	0.161	2.7	1.8	2.0
1.5.79	2.0	3.2	5.6	0.178	0.174	0.166	2.8	1.7	1.8
9.5.79	1.1	2.4	5.8	0.175	0.171	0.168	3.4	2.2	2.3
16.5.79	1.1	2.3	4.3	0.172	0.168	0.164	4.6	3.1	3.2
23.5.79	1.0	2.2	3.3	0.140	0.152	0.159	6.4	4.7	3.6
29.5.79	1.2	2.2	3.1	0.082	0.128	0.123	8.5	5.3	5.5
7.6.79	1.4	2.7	1.9	0.170	0.160	0.155	5.8	4.9	4.4
13.6.79	0.7	1.4	1.2	0.168	0.172	0.154	5.6	4.3	4.8
28.6.79	0.5	0.9	0.8	0.195	0.196	0.197	2.0	2.1	1.7
5.7.79	0.4	0.5	0.9	0.203	0.207	0.205	1.8	1.4	1.5
12.7.79	0.4	0.6	0.8	0.206	0.204	0.204	1.0	1.1	1.0

Treatment Means at the 45cm Depth

Date	Nitrous Oxide			Oxygen			Carbon Dioxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	($ml\ ml^{-1} \times 10^{6}$)			($ml\ ml^{-1}$)			($ml\ ml^{-1} \times 10^{2}$)		
5.9.78	3.7	3.9	2.1	0.145	0.103	0.163	4.6	4.7	2.7
8.9.78	5.3	4.5	17.8	0.138	0.137	0.151	4.7	3.3	4.4
12.9.78	3.0	3.9	23.6	0.151	0.087	0.173	4.6	7.7	2.7
19.9.78	1.9	13.3	13.1	0.170	0.118	0.187	4.0	6.3	3.0
25.9.78	1.2	5.6	4.3	0.179	0.176	0.188	3.6	2.8	2.1
2.10.78	3.0	31.2	7.5	0.140	0.120	0.164	3.6	4.3	2.1
9.10.78	2.0	16.6	4.5	0.159	0.131	0.189	4.4	5.4	2.3
16.10.78	1.2	4.5	2.7	0.168	0.164	0.181	4.1	3.0	2.5
23.10.78	0.7	2.1	1.4	0.180	0.174	0.191	3.1	3.3	2.0
30.10.78	1.0	2.2	0.6	0.179	0.182	0.207	3.0	3.0	0.6
6.11.78	3.9	5.9	2.2	0.137	0.147	0.185	3.9	3.7	1.5
20.11.78	1.9	6.6	4.3	0.100	0.105	0.150	3.4	2.9	1.3
27.11.78	1.1	4.8	3.5	0.153	0.111	0.129	3.0	3.2	2.6
3.12.78	0.9	1.5	2.2	0.156	0.150	0.138	3.5	2.2	2.6
11.12.78	1.2	1.7	2.4	0.153	0.170	0.191	2.0	1.6	1.2
18.12.78	0.7	2.1	1.0	0.173	0.155	0.150	2.1	1.9	1.4
3.1.79	1.4	3.2	2.0	0.168	0.163	0.169	1.8	1.7	0.8
8.1.79	8.2	4.1	2.2	0.137	0.183	0.163	2.8	1.6	1.1
15.1.79	4.6	8.8	7.4	0.158	0.172	0.149	2.6	1.6	2.3
22.1.79	1.0	1.6	0.8	0.192	0.184	0.199	1.4	0.6	0.4
29.1.79	4.0	5.5	4.0	0.160	0.186	0.160	2.2	0.9	1.3
6.2.79	6.5	8.2	9.3	0.149	0.162	0.164	2.2	1.0	1.6

CONTINUED/

Treatment Means at the 45cm Depth (CONTINUED)

Date	Nitrous Oxide			Oxygen			Carbon Dioxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x 10^{6}			ml ml^{-1}			ml ml^{-1} x 10^{2}		
14.2.79	10.8	15.2	8.9	0.151	0.159	0.159	1.6	1.0	1.2
21.2.79	16.8	58.0	93.7	0.151	0.126	0.086	1.9	1.6	2.8
28.2.79	72.2	67.4	98.5	0.138	0.151	0.110	2.4	1.8	4.8
7.3.79	50.9	34.1	177.7	0.148	0.155	0.115	3.5	3.4	5.2
15.3.79	28.5	28.5	84.8	0.187	0.127	0.130	1.7	2.8	3.6
23.3.79	53.0	24.0	106.7	0.167	0.166	0.130	1.9	1.9	5.5
28.3.79	23.6	5.7	64.1	0.157	0.183	0.140	2.2	1.5	5.1
6.4.79	9.3	6.5	11.8	0.179	0.152	0.210	2.4	1.4	1.9
12.4.79	2.2	3.7	3.7	0.193	0.152	0.206	1.0	1.2	0.1
19.4.79	8.4	10.9	24.5	0.129	0.094	0.138	5.0	2.9	4.5
25.4.79	3.5	8.7	30.3	0.127	0.113	0.093	5.3	2.9	4.8
1.5.79	2.5	9.4	26.0	0.137	0.114	0.099	4.9	3.2	3.5
9.5.79	2.1	4.7	23.3	0.131	0.100	0.061	5.3	3.7	4.1
16.5.79	1.8	3.3	26.8	0.122	0.101	0.052	7.5	4.8	6.1
23.5.79	1.7	3.1	20.5	0.095	0.095	0.047	8.8	5.6	7.3
29.5.79	1.1	4.7	13.7	0.080	0.088	0.092	9.2	6.2	5.8
7.6.79	1.3	9.3	14.0	0.124	0.097	0.069	9.2	7.7	8.5
13.6.79	1.4	7.5	9.4	0.122	0.081	0.055	7.6	7.3	8.2
28.6.79	0.7	1.1	1.9	0.179	0.171	0.142	3.1	2.4	2.7
5.7.79	0.5	0.7	0.8	0.198	0.204	0.202	2.2	1.5	1.1
12.7.79	0.6	1.0	0.9	0.202	0.200	0.202	1.6	1.5	1.0

B. Analysis of Variance over 4 time periods.

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
1.	Treatments	2	1.06	0.53	20.3***	2	14.7	7.4	26.4***
	(difference bet.control & others					1	14.5	14.5	51.9***)
	(difference bet.slurried & others	1	0.89	0.89	34.4***				)
	(difference bet.control & fertilised	1	0.16	0.16	6.3*				)
	Depths	2	0.15	0.08	2.9	2	0.86	0.4	1.5
	(difference bet. 15cm & others	1	0.15	0.15	5.8*				)
	Treatment x Depth	4	0.06	0.02	0.6	4	0.69	0.2	0.6
	Error	27	0.72	0.03		27	7.69	0.3	
	Total	35	1.99			35	23.98		
2.	Treatments	2	0.18	0.09	1.5	2	1.84	0.92	1.61
	Depths	2	0.31	0.16	2.6	2	2.56	1.28	2.25
	(difference bet. 30cm & others	1	2.46	2.46	4.3*				)
	(difference bet. 45cm & others					1	0.28	0.28	4.7*)
	Treatment x Depth	4	0.18	0.05	0.8	4	1.03	0.26	0.5
	Error	27	1.63	0.06		27	15.36	0.57	
	Total	35	2.30			35	20.78		

CONTINUED/

B. Analysis of Variance over 4 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
3.	Treatments	2	0.07	0.04	0.5	2	2.92	1.46	2.27
	(difference bet.fertilised & others					1	2.76	2.76	4.29*)
	Depths	2	0.74	0.37	5.5**	2	5.18	2.59	4.03*
	(difference bet. 15cm & others	1	0.61	0.61	9.1**	1	5.17	5.17	8.05**)
	Treatment x Depth	4	0.45	0.11	1.7	4	0.37	0.09	0.15
	Error	27	1.82	0.07		27	17.35	0.64	
	Total	35	3.08			35	25.83		
4.	Treatments	2	0.06	0.03	1.1	2	7.80	3.89	12.7 ***
	(difference bet.control & others					1	6.44	6.44	21.1 ***)
	(difference bet.slurry & fertilised					1	1.31	1.31	4.3*)
	Depths	2	3.19	1.59	61.3	2	8.30	4.15	13.6***
	difference bet.45cm & others	1	3.01	3.01	115.7	1	7.26	7.26	23.8***
	difference bet.15cm & 30cm.	1	0.18	0.18	6.8*				
	Treatment x Depth	4	0.10	0.02	0.92	4	3.01	0.75	2.46
	Error	27	0.69	0.03		27	8.24	0.31	
	Total	35	4.03			35	27.33		

CONTINUED/

B. Analysis of Variance over 4 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
Means over all periods	Treatments	2	0.028	0.014	0.53	2	4.17	2.09	8.11**
	(Control different from other					1	3.93	3.93	15.27***)
	Depths	2	0.725	0.362	13.61***	2	3.33	1.67	6.48**
	(15cm different from other					1	3.32	3.32	12.92**)
	(45cm different from other	1	0.601	0.601	22.61***				)
	(30cm different from 15cm	1	0.123	0.123	4.64*				)
	Treatment x Depth	4	0.097	0.024	0.90	4	0.14	0.04	0.14
	Error	27	0.718	0.027		27	6.95	0.26	
	Total	35	1.568			35	14.59		
All periods combined	Total (Treatment & Depth)	35	6.07			35	59.16		
	Time	3	0.36	0.12	4.01*	3	79.36	26.45	116.05***
	Time x Treatment	6	1.17	0.19	6.51***	6	6.61	1.10	4.83***
	Time x Depth	6	1.38	0.23	7.68***	6	7.96	1.33	5.82***
	Error	93	2.78	0.03		93	21.20	0.23	
	Total	143	11.76			143	174.29		

Notes: Sum of squares were partitioned into independent contrasts by a method described by Pearce (1965).

APPENDIX 8: A. DATA FOR CHAPTER 3

Means at 15cm

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x10^{2}			ml ml^{-1}			ml ml^{-1} x10^{6}		
5.7.79	0.82	0.84	0.76	0.209	0.209	0.209	0.4	0.3	0.4
12.7.79	0.52	0.61	0.60	0.207	0.209	0.206	0.5	0.4	0.5
17.7.79	0.72	0.86	0.66	0.206	0.207	0.206	0.6	0.8	0.6
19.7.79	0.53	0.58	0.50	0.203	0.204	0.204	0.4	0.5	0.4
23.7.79	0.57	0.58	0.48	0.209	0.209	0.210	0.6	0.5	0.7
26.7.79	0.60	0.63	0.49	0.209	0.209	0.208	0.5	0.4	0.5
2.8.79	0.47	0.50	0.43	0.206	0.205	0.204	0.3	0.3	0.3
8.8.79	1.01	0.52	0.57	0.206	0.206	0.205	0.3	0.3	0.3
15.8.79	1.01	0.80	0.98	0.202	0.204	0.199	0.6	0.6	0.7
22.8.79	1.47	1.65	1.38	0.201	0.184	0.199	0.4	1.7	0.8
24.8.79	1.63	1.58	1.36	0.197	0.191	0.196	0.5	2.6	0.8
30.8.79	1.32	1.45	0.88	0.199	0.197	0.202	0.4	0.5	0.5
6.9.79	1.26	1.26	1.23	0.199	0.202	0.206	0.3	0.3	0.3
13.9.79	0.88	0.73	0.75	0.204	0.206	0.204	0.3	0.3	0.3
19.9.79	0.84	0.75	0.70	0.202	0.204	0.206	0.3	0.4	0.4
26.9.79	0.96	0.90	0.67	0.206	0.204	0.207	0.3	0.3	0.3
3.10.79	0.67	0.68	0.47	0.207	0.205	0.207	0.4	0.3	0.4
10.10.79	1.45	1.20	0.95	0.195	0.194	0.201	0.7	0.8	0.7
17.10.79	2.06	1.38	1.36	0.178	0.181	0.186	1.7	2.1	1.4
24.10.79	1.93	1.15	1.21	0.189	0.192	0.198	0.8	0.5	0.4

CONTINUED/

***NB. All means were calculated using transformed data. The concentrations below are the reverse transform of the mean.

Means at 15cm (CONTINUED)

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml $ml^{-1} \times 10^{2}$			ml ml^{-1}			ml $ml^{-1} \times 10^{6}$		
31.10.79	1.96	1.12	1.26	0.180	0.182	0.192	1.1	1.6	0.9
1.11.79	2.20	1.61	1.13	0.178	0.168	0.194	1.1	2.9	1.5
5.11.79	1.82	1.16	0.93	0.186	0.190	0.201	2.0	28.5	6.1
7.11.79	2.00	1.22	1.11	0.176	0.182	0.194	1.8	27.5	7.6
14.11.79	1.73	0.75	0.86	0.191	0.195	0.201	1.2	3.4	4.2
21.11.79	1.63	0.66	0.69	0.185	0.193	0.204	3.1	9.1	9.7
28.11.79	1.51	0.60	0.78	0.180	0.191	0.193	1.0	3.4	7.4
5.12.79	1.08	0.35	0.61	0.203	0.190	0.203	1.5	2.4	11.2
12.12.79	1.56	0.73	0.98	0.187	0.172	0.189	0.4	0.6	6.4
19.12.79	1.76	0.57	0.89	0.172	0.191	0.196	0.5	1.6	5.4
31.12.79	1.48	0.80	0.64	0.176	0.191	0.203	1.2	2.8	13.2
9.1.80	1.31	0.59	0.49	0.173	0.177	0.197	5.6	17.3	11.7
16.1.80	1.71	0.46	0.68	0.153	0.187	0.190	3.3	1.5	2.9
23.1.80	0.94	0.32	0.78	0.163	0.182	0.175	5.7	4.6	5.4
30.1.80	1.15	0.21	0.84	0.168	0.195	0.185	4.2	2.7	4.0
6.2.80	0.83	0.28	0.64	0.185	0.202	0.192	2.2	1.5	2.0
13.2.80	1.66	0.52	0.75	0.169	0.191	0.198	0.8	1.9	1.2
20.2.80	2.10	0.56	0.85	0.163	0.199	0.198	1.8	1.1	1.0
27.2.80	1.54	0.55	0.72	0.180	0.201	0.195	1.4	0.8	0.7
5.3.80	1.25	0.49	0.69	0.187	0.194	0.206	1.2	0.7	0.4
11.3.80	1.67	0.76	0.64	0.181	0.201	0.205	1.4	1.0	0.6
24.3.80	1.69	0.63	0.98	0.158	0.192	0.187	1.6	1.0	1.1

CONTINUED/

Means at 15cm (CONTINUED)

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml $ml^{-1} \times 10^{2}$			ml ml^{-1}			ml $ml^{-1} \times 10^{6}$		
2.4.80	1.87	1.11	0.88	0.176	0.177	0.198	0.8	0.8	0.6
11.4.80	1.56	1.03	0.81	0.200	0.194	0.204	0.7	0.7	0.5
16.4.80	1.36	1.41	0.99	0.191	0.191	0.204	0.5	1.1	0.6
18.4.80	0.88	1.51	0.81	0.182	0.180	0.187	0.6	2.4	0.6
22.4.80	1.10	1.05	0.63	0.200	0.200	0.208	0.7	0.9	0.4
25.4.80	1.11	1.17	0.76	0.202	0.203	0.209	0.3	0.7	0.4
30.4.80	0.95	0.87	0.64	0.205	0.205	0.206	0.3	0.4	0.3
8.5.80	0.75	0.54	0.50	0.210	0.210	0.210	0.4	0.5	0.5
14.5.80	0.73	0.61	0.60	0.203	0.201	0.205	0.3	0.3	0.3
22.5.80	0.45	0.37	0.33	0.209	0.209	0.210	0.4	0.4	0.4
28.5.80	0.51	0.50	0.38	0.207	0.209	0.208	0.5	0.7	0.5
4.6.80	0.58	0.50	0.44	0.208	0.208	0.209	0.4	0.5	0.6
11.6.80	1.11	0.60	0.67	0.203	0.206	0.205	0.5	0.5	0.6
18.6.80	1.50	1.07	1.27	0.197	0.203	0.195	0.7	0.5	0.7
27.6.80	1.14	0.61	0.89	0.201	0.199	0.204	0.5	0.6	0.7
2.7.80	1.64	0.77	1.31	0.198	0.208	0.200	0.6	0.4	0.6

Means at 30cm

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml $ml^{-1} \times 10^{2}$			ml ml^{-1}			ml $ml^{-1} \times 10^{6}$		
5.7.79	1.43	1.52	1.50	0.208	0.203	0.205	0.6	0.5	0.4
12.7.79	1.05	1.14	0.98	0.205	0.202	0.206	0.5	0.4	0.5
17.7.79	0.90	1.47	0.88	0.208	0.200	0.204	0.6	0.9	0.4
19.7.79	0.89	1.25	0.72	0.200	0.196	0.206	0.5	0.7	0.4
23.7.79	0.82	0.98	0.70	0.206	0.208	0.209	0.6	0.7	0.5
26.7.79	0.90	1.06	0.73	0.207	0.207	0.208	0.6	0.6	0.5
2.8.79	0.82	0.98	0.64	0.201	0.202	0.206	0.4	0.3	0.3
8.8.79	0.80	0.92	0.72	0.204	0.200	0.204	0.3	0.3	0.3
15.8.79	1.14	1.12	0.97	0.200	0.198	0.202	0.9	0.7	0.5
22.8.79	1.40	2.53	1.72	0.190	0.177	0.193	1.0	2.2	1.1
24.8.79	2.02	2.60	1.81	0.188	0.179	0.194	0.9	4.2	1.0
30.8.79	1.91	2.11	1.48	0.193	0.192	0.199	0.7	0.9	0.6
6.9.79	1.72	1.85	1.26	0.193	0.196	0.203	0.3	0.4	0.3
13.9.79	1.31	1.55	0.91	0.200	0.196	0.206	0.4	0.4	0.3
19.9.79	1.02	1.33	0.95	0.201	0.199	0.203	0.4	0.4	0.4
26.9.79	1.21	1.23	0.88	0.202	0.202	0.208	0.4	0.4	0.3
3.10.79	1.20	1.12	0.68	0.199	0.198	0.205	1.0	0.3	0.3
10.10.79	2.12	1.90	1.19	0.186	0.188	0.200	1.4	1.3	0.8
17.10.79	2.34	2.26	1.69	0.166	0.170	0.183	1.8	2.6	1.5
24.10.79	2.27	2.14	1.45	0.186	0.185	0.193	0.9	1.0	0.8
31.10.79	2.26	2.89	1.59	0.178	0.151	0.187	1.2	1.9	0.8
1.11.79	2.07	3.26	1.65	0.175	0.147	0.185	0.9	4.2	2.0
5.11.79	1.73	2.18	1.09	0.193	0.185	0.191	1.7	13.7	5.0

CONTINUED/

Means at 30cm (CONTINUED)

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x10^{2}			ml ml^{-1}			ml ml^{-1} x10^{6}		
7.11.79	1.58	3.12	1.35	0.186	0.158	0.191	1.8	15.3	11.2
14.11.79	1.61	2.29	1.27	0.185	0.187	0.192	1.0	4.1	6.6
21.11.79	1.17	2.15	0.99	0.200	0.183	0.187	1.8	8.4	31.4
28.11.79	1.24	2.17	0.90	0.192	0.177	0.185	0.9	2.0	31.3
5.12.79	1.03	1.57	0.66	0.195	0.195	0.195	2.1	2.7	49.0
12.12.79	1.40	1.07	0.63	0.184	0.197	0.199	1.1	0.5	39.6
19.12.79	1.46	1.82	1.12	0.183	0.201	0.163	1.0	1.2	63.2
31.12.79	1.02	1.03	0.73	0.196	0.192	0.185	4.5	3.4	13.5
9.1.80	1.69	1.10	0.65	0.172	0.179	0.174	14.7	2.3	28.1
16.1.80	0.82	1.64	0.70	0.188	0.175	0.171	1.0	0.8	14.7
23.1.80	0.50	1.68	0.71	0.180	0.150	0.164	4.1	1.9	17.2
30.1.80	0.70	1.66	0.55	0.195	0.181	0.201	2.3	3.4	3.1
6.2.80	0.70	2.19	0.83	0.199	0.173	0.196	1.0	2.1	3.3
13.2.80	0.76	1.63	1.01	0.204	0.192	0.193	1.0	0.7	5.2
20.2.80	1.57	2.26	1.56	0.179	0.133	0.182	2.3	1.2	6.3
27.2.80	1.34	2.16	1.18	0.197	0.138	0.193	1.6	1.4	3.1
5.3.80	1.21	2.09	1.18	0.197	0.128	0.173	1.2	2.5	2.0
11.3.80	1.65	1.93	1.28	0.194	0.159	0.190	1.7	0.9	2.3
24.3.80	1.52	2.14	1.12	0.183	0.176	0.181	2.3	1.3	2.7
2.4.80	1.26	2.44	1.87	0.199	0.121	0.173	0.9	3.1	3.0
11.4.80	1.89	2.80	2.04	0.191	0.124	0.188	0.9	3.3	1.5
16.4.80	1.85	2.91	1.86	0.190	0.126	0.185	0.8	4.5	1.3

CONTINUED/

Means at 30cm (CONTINUED)

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml $ml^{-1} \times 10^{2}$			ml ml^{-1}			ml $ml^{-1} \times 10^{6}$		
18.4.80	1.60	3.29	1.56	0.181	0.103	0.175	1.0	6.1	1.1
22.4.80	1.16	2.66	1.21	0.197	0.140	0.196	0.6	5.6	0.9
25.4.80	1.62	2.78	1.37	0.200	0.130	0.196	0.5	4.3	0.7
30.4.80	1.48	1.83	1.04	0.199	0.166	0.202	0.4	2.2	0.5
8.5.80	1.03	1.13	0.77	0.209	0.205	0.207	0.5	1.0	0.6
14.5.80	1.08	0.88	0.98	0.203	0.199	0.201	0.3	0.4	0.3
22.5.80	0.70	0.69	0.56	0.207	0.203	0.209	0.4	0.4	0.4
28.5.80	0.75	0.58	0.58	0.202	0.207	0.208	0.9	0.7	0.6
4.6.80	0.79	0.88	0.68	0.209	0.207	0.208	0.5	0.5	0.6
11.6.80	1.11	0.97	1.04	0.202	0.204	0.203	0.6	0.5	0.6
18.6.80	1.67	1.51	1.37	0.193	0.198	0.196	0.6	0.6	0.7
27.6.80	1.29	1.13	1.50	0.197	0.198	0.198	0.7	0.6	0.8
2.7.80	1.80	1.45	1.50	0.198	0.199	0.196	0.6	0.6	0.7

Means at 45cm.

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x10^{2}			ml ml^{-1}			ml ml^{-1} x10^{6}		
5.7.79	1.82	1.58	1.83	0.202	0.202	0.201	0.8	0.7	0.7
12.7.79	1.37	1.35	1.33	0.202	0.201	0.201	0.7	0.8	0.8
17.7.79	1.17	1.47	1.04	0.201	0.195	0.202	0.7	1.4	0.8
19.7.79	1.19	1.22	0.99	0.200	0.195	0.201	0.6	0.8	0.5
23.7.79	1.03	1.15	0.88	0.206	0.207	0.208	0.7	0.8	0.8
26.7.79	1.10	1.26	0.96	0.202	0.206	0.206	0.7	0.8	0.5
2.8.79	0.98	1.17	0.99	0.203	0.201	0.204	0.5	0.4	0.4
8.8.79	0.96	1.01	0.87	0.204	0.203	0.203	0.3	0.3	0.4
15.8.79	1.16	1.15	1.05	0.200	0.201	0.201	0.8	1.1	1.3
22.8.79	1.97	2.37	1.76	0.187	0.176	0.192	1.1	1.9	1.6
24.8.79	2.01	2.68	2.09	0.189	0.180	0.188	0.8	4.0	1.4
30.8.79	1.69	2.37	1.73	0.192	0.187	0.197	0.7	1.2	0.7
6.9.79	1.34	1.88	1.15	0.200	0.198	0.202	0.3	0.3	0.4
13.9.79	1.29	1.39	1.24	0.199	0.199	0.202	0.4	0.5	0.4
19.9.79	1.10	1.33	0.90	0.200	0.198	0.202	0.4	0.5	0.4
26.9.79	1.15	1.23	0.83	0.203	0.202	0.207	0.4	0.5	0.5
3.10.79	1.07	1.20	0.79	0.201	0.198	0.201	0.4	0.5	0.6
10.10.79	1.74	1.96	1.28	0.190	0.180	0.195	1.2	1.5	1.2
17.10.79	2.02	1.82	1.39	0.172	0.173	0.181	1.6	3.0	2.2
24.10.79	1.88	2.10	1.42	0.185	0.182	0.192	0.7	0.9	0.8
31.10.79	2.11	2.22	1.34	0.180	0.161	0.186	1.1	2.5	1.2
1.11.79	1.96	2.43	1.19	0.187	0.143	0.192	0.8	1.8	1.2
5.11.79	1.86	2.49	0.75	0.185	0.165	0.171	1.9	11.1	1.5

CONTINUED/

Means at 45cm (CONTINUED)

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x10^{2}			ml ml^{-1}			ml ml^{-1} x10^{6}		
7.11.79	1.43	2.62	0.78	0.176	0.153	0.181	0.8	10.0	2.6
14.11.79	2.22	2.06	0.67	0.178	0.159	0.194	0.4	0.6	1.4
21.11.79	1.73	1.91	0.39	0.184	0.176	0.203	1.2	4.8	12.4
28.11.79	2.17	1.60	0.73	0.181	0.188	0.149	0.6	1.5	6.3
5.12.79	2.85	3.26	0.73	0.164	0.132	0.167	2.2	3.8	22.0
12.12.79	3.34	3.53	0.91	0.164	0.173	0.079	0.5	2.1	112.8
19.12.79	2.53	1.69	0.76	0.166	0.198	0.199	0.6	1.0	24.9
31.12.79	1.52	0.66	0.78	0.187	0.202	0.168	0.9	2.5	16.9
9.1.80	0.60	1.19	0.45	0.189	0.190	0.085	4.7	6.6	47.3
16.1.80	1.13	0.95	0.66	0.184	0.188	0.148	0.7	1.6	14.2
23.1.80	1.80	1.17	0.72	0.163	0.195	0.165	1.8	3.4	9.6
30.1.80	1.33	1.78	0.39	0.200	0.183	0.199	1.8	5.3	3.4
6.2.80	1.14	1.96	0.85	0.195	0.166	0.199	1.1	6.0	3.9
13.2.80	2.45	2.68	0.93	0.144	0.127	0.180	0.5	8.2	6.3
20.2.80	2.08	1.92	1.20	0.146	0.160	0.165	0.6	1.2	5.1
27.2.80	1.68	2.12	0.82	0.186	0.188	0.173	0.9	1.5	3.0
5.3.80	2.00	1.47	0.86	0.163	0.143	0.175	4.2	2.6	3.6
11.3.80	1.58	1.18	0.48	0.168	0.190	0.194	1.0	2.2	2.5
24.3.80	2.11	1.41	0.86	0.128	0.146	0.159	2.0	3.8	3.6
2.4.80	2.00	2.41	1.29	0.150	0.103	0.160	1.0	3.3	3.0

CONTINUED/

Means at 45cm (CONTINUED)

Date	Carbon Dioxide			Oxygen			Nitrous Oxide		
	Control	Slurried	Fertilised	Control	Slurried	Fertilised	Control	Slurried	Fertilised
	ml ml^{-1} x10^{2}			ml ml^{-1}			ml ml^{-1} x10^{6}		
11.4.80	2.44	1.63	1.41	0.149	0.128	0.170	1.7	3.3	2.6
16.4.80	2.16	1.82	1.55	0.153	0.150	0.158	1.6	4.3	2.2
18.4.80	2.05	2.32	1.35	0.143	0.110	0.159	2.0	8.9	2.2
22.4.80	1.88	1.64	1.18	0.181	0.136	0.174	0.8	9.3	1.5
25.4.80	1.74	1.74	1.12	0.169	0.126	0.176	1.3	8.6	1.3
30.4.80	1.83	1.41	1.01	0.162	0.136	0.176	1.1	7.1	0.8
8.5.80	1.59	1.07	0.91	0.176	0.140	0.190	0.9	5.6	0.8
14.5.80	1.52	1.02	0.85	0.173	0.130	0.185	0.4	2.1	0.6
22.5.80	0.99	0.86	0.66	0.203	0.191	0.207	0.5	0.8	0.5
28.5.80	0.82	0.54	0.67	0.205	0.199	0.204	0.6	0.8	0.8
4.6.80	0.98	0.97	0.75	0.207	0.205	0.207	0.5	0.6	0.9
11.6.80	1.27	1.31	1.07	0.200	0.198	0.203	0.5	0.7	1.0
18.6.80	1.58	1.39	1.23	0.190	0.193	0.197	0.6	0.7	1.0
27.6.80	1.92	1.69	1.32	0.189	0.195	0.199	0.6	0.9	0.6
2.7.80	1.77	1.47	1.46	0.196	0.194	0.200	0.6	0.6	0.8

B. Analysis of Variance for 8 time periods.

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
1.	Treatment	2	1.64	0.82	5.14*	2	7.89	3.94	11.94*
	(Slurry different from others	1	1.64	1.64	10.28*	1	7.29	7.29	22.10**)
	Blocks	2	0.72	0.36	2.25	2	0.81	0.40	1.21
	Error	4	0.64	0.16		4	1.32	0.33	
	Total	8	3.00			8	10.02		
	Depth	2	4.34	2.17	7.73**	2	6.17	3.09	5.13*
	(45cm different from others	1	3.69	3.69	13.17***	1	5.84	5.84	10.05**)
	Depth x Treatment	4	2.04	0.51	1.82	4	1.68	0.41	0.71
	Depth x Blocks					4	0.78	0.19	0.34
	Depth x Blocks x Treatments					8	4.55	0.57	0.98
	Error	39	10.95	0.28		27	15.69	0.58	
	Total	53	20.33			53	38.89		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
2.	Treatment	2	0.34	0.17	1.84	2	0.67	0.33	0.57
	Blocks	2	0.68	0.34	3.68	2	12.64	6.32	10.90*
	(Block 2 different from others					1	12.63	12.63	21.78**)
	Error	4	0.37	0.09		4	2.30	0.58	
	Total	8	1.39			8	15.61		
	Depth	2	2.57	1.28	11.72***	2	8.65	4.33	11.29***)
	(15cm different from others	1	2.40	2.40	21.95***				
	(45cm different from others					1	8.13	8.13	21.21***)
	Depth x Treatment	4	0.77	0.19	1.74	4	2.98	0.75	1.96
	Depth x Block					4	2.56	0.64	1.67
	Depth x Block x Treatment					8	2.99	0.37	0.97
	Error	39	4.26	0.11		27	10.35	0.38	
	Total	53	8.99			53	43.14		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
3.	Treatment	2	3.37	1.68	20.42**	2	26.01	13.00	27.37**
	(Slurry different from others	1	3.34	3.34	40.47**	1	23.07	23.07	48.57**)
	Blocks	2	0.63	0.32	3.82	2	3.11	1.55	3.27
	Error	4	0.33	0.08		4	1.90	0.48	
	Total	8	4.14			8	31.02		
	Depth	2	2.35	1.17	12.93***	2	6.48	3.44	9.42***
	(15cm different from others	1	2.32	2.32	25.63***	1	6.35	6.35	17.39***)
	Depth x Treatment	4	.28	0.07	0.77	4	2.07	0.52	1.42
	Depth x Block					4	1.70	0.43	1.18
	Depth x Block x Treatment					8	2.93	0.37	1.01
	Error	39	3.53	0.09		27	9.86	0.37	
	Total	53	10.30			53	54.06		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
4.	Treatment	2	2.55	1.28	14.17*	2	0.31	0.15	0.75
	(Fertiliser different from others	1	2.54	2.54	28.18**				)
	Blocks	2	0.09	0.05	0.50	2	1.34	0.67	3.22
	Error	4	0.36	0.09		4	0.83	0.21	
	Total	8	2.78			8	2.48		
	Depth	2	1.95	0.98	9.93***	2	6.67	3.33	15.50***
	(15 cm different from others	1	1.93	1.93	19.73***	1	6.59	6.59	31.38***)
	Depth x Treatment	4	0.31	0.08	0.79	4	2.13	0.53	2.47
	Depth x Block					4	0.47	0.12	0.56
	Depth x Block x Treatment					8	1.69	0.21	0.98
	Error	39	3.83	0.10		27	5.80	0.21	
	Total	53	8.87			53	19.24		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2 df	O_2 ss	O_2 ms	O_2 F	N_2O df	N_2O ss	N_2O ms	N_2O F
5.	Treatment	2	6.17	3.08	11.87*	2	11.24	5.62	12.85*
	(Slurry different from others	1	4.96	4.96	19.07*	1	10.83	10.83	24.61**)
	Blocks	2	0.03	0.01	0.06	2	4.07	2.03	4.64
	Error	4	0.26	0.06		4	1.75	0.44	
	Total	8	6.46			8	17.06		
	Depth	2	0.77	0.39	1.18	2	0.29	0.14	0.54
	Depth x Treatment	4	0.64	0.16	0.49	4	0.45	0.11	0.43
	Depth x Block					4	0.91	0.23	0.89
	Depth x Block x Treatment					8	1.16	0.15	0.58
	Error	37	12.04	0.33		25	6.43	0.26	
	Total	51	19.91			51	26.30		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
6.	Treatment	2	2.02	1.01	2.48	2	16.07	8.03	3.60
	Blocks	2	3.48	1.74	4.27	2	0.67	0.34	0.15
	Error	4	1.63	0.41		4	8.94	2.24	
	Total	8	7.13			8	25.68		
	Depth	2	3.24	1.62	2.14	2	0.70	0.35	0.23
	(15cm different from others	1	2.48	2.48	3.26*				)
	Depth x Treatment	4	2.83	0.71	0.94	4	3.32	0.83	0.54
	Depth x Block					4	0.45	0.11	0.07
	Depth x Block x Treatment					8	8.25	1.03	0.67
	Error	38	28.83	0.76		26	40.01	1.54	
	Total	52	42.02			52	78.41		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
7.	Treatments	2	8.21	4.10	27.33**	2	46.28	23.14	23.86**
	(Slurry different from others	1	7.63	7.63	50.85**	1	45.33	45.33	46.73**)
	Blocks	2	4.34	2.17	14.47*	2	9.92	4.96	5.11
	(Block 3 different from others	1	3.96	3.96	26.40**	1	9.91	9.91	10.22*)
	Error	4	0.61	0.15		4	3.87	0.97	
	Total	8	13.16			8	60.07		
	Depth	2	26.71	13.36	20.92***	2	41.74	20.87	26.53***
	(15cm different from others	1	22.56	22.56	35.24***	1	35.25	35.25	44.81***)
	(30cm different from 45cm	1	4.15	4.15	6.48*	1	6.48	6.48	8.24**)
	Depth x Treatment	4	3.50	0.88	1.37	4	2.80	0.70	0.89
	Depth x Block					4	4.06	1.02	1.30
	Depth x Block x Treatment					8	4.97	0.62	0.79
	Error	36	22.98	0.64		24	18.88	0.79	
	Total	50	66.35			50	132.52		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2 df	O_2 ss	O_2 ms	O_2 F	N_2O df	N_2O ss	N_2O ms	N_2O F
8.	Treatments	2	1.15	0.57	7.13*	2	1.73	0.86	17.20*)
	(Fertiliser different from others	1	1.09	1.09	13.58**	1	1.73	1.73	34.76**)
	(Control different from others					2	0.82	0.41	8.2*
	Blocks	2	0.62	0.31	3.88	1	0.81	0.81	16.19)
	(Block 2 different from others								
	Error	4	0.32	0.08		4	0.19	0.05	
	Total	8	2.09			8	2.74		
	Depth	2	12.07	6.03	66.04***	2	8.56	4.28	35.92***
	(45cm different from others	1	10.19	10.19	111.54***	1	7.29	7.29	61.17***)
	(30cm different from 15cm	1	1.87	1.87	20.54***	1	1.28	1.28	10.74**)
	Depth x Treatment	4	0.85	9.30	1.18	4	1.17	0.29	2.43
	Depth x Block					4	0.79	0.20	1.68
	Depth x Block x Treatment					8	1.49	0.19	1.59
	Error	36	3.29	0.09		24	2.86	0.12	
	Total	50	27.54			51	17.61		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O			
		df	ss	ms	F	df	ss	ms	F
All periods combined	Main Plots	8	22.19			8	53.15		
	Sub Plots	45	66.82			45	96.11		
	Total	53	89.01			53	149.26		
	Time	7	169.98	24.28	99.02***	7	332.57	47.51	120.58***
	Time x Treatment	14	7.05	0.50	2.04*	14	65.51	4.68	11.88***
	Time x Depth	14	18.01	1.29	5.26***	14	31.81	2.27	5.77***
	Time x Depth x Treatment	28	5.97	0.21	0.86	28	13.79	0.49	1.25
	Error	306	75.03	0.25		307	120.94	0.39	
	Total	422	365.05			423	713.88		

CONTINUED/

B. Analysis of Variance for 8 time periods (CONTINUED)

Period	Source	O_2				N_2O				
		df	ss	ms	F	df	ss	ms	F	
Means whole period	Treatments	2	1.63	0.81	15.32*	2	3.94	1.97	2.29	
	(Control different from others					1	3.94	3.94	4.58	)
	(Fertilised different from others	1	1.33	1.33	25.16**					)
	Blocks	2	0.74	0.37	6.98*	2	0.74	0.37		
	(Block 3 different from others	1	0.71	0.71	13.47*					
	Error	4	0.21	0.05		4	3.44	0.86		
	Total	8	2.58			8	8.12			
	Depths	2	4.40	2.20	11.40***	2	4.43	2.21	7.13**	
	(15cm different from others	1	3.77	3.77	19.56***	1	4.17	4.17	13.44**	)
	Depth x Treatment	4	0.86	0.21	1.11	4	1.17	0.29	0.94	
	Depth x Block					4	0.27	0.07	0.22	
	Depth x Block x Treatment					8	1.74	0.22	0.70	
	Error	39	7.55	0.19		27	8.38	0.31		
	Total	53	15.38			53	24.11			

NOTES: Missing values were fitted by setting residual error to zero and total degrees of freedom reduced by 1 for each fitted value.

APPENDIX 9 FLUX DATA FOR CHAPTER 8

N.B. Means were calculated using transformed data. Fluxes in the table below are the reverse transforms of the means

Date	Flux ($gN\ ha^{-1}\ h^{-1}$)					
	Control Plots		Slurried Plots		Fertilised Plots	
	$-C_2H_2$	$+C_2H_2$	$-C_2H_2$	$+C_2H_2$	$-C_2H_2$	$+C_2H_2$
28. 4.81	1.7	1.0	0.9	0.6	0.6	1.2
30. 4.81	0.1	-0.2	-0.1	0.0	-0.4	0.1
5. 5.81	0.7	1.3	1.0	1.6	0.7	0.8
12. 5.81	-0.4	0.6	-0.3	1.3	1.5	0.5
21. 5.81	0.4	4.0	0.0	2.1	0.1	2.5
28. 5.81	0.0	0.2	0.2	-0.4	-0.2	0.1
4. 6.81	0.6	1.1	0.5	1.0	0.2	0.9
10. 6.81	1.2	3.4	0.7	1.4	0.7	1.8
2. 7.81	0.7	0.7	0.6	1.0	-0.1	0.3
7. 7.81	1.2	0.6	2.4	8.1	0.6	0.5
8. 7.81	0.7	0.4	0.8	2.6	2.5	0.6
9. 7.81	0.8	1.1	1.4	2.2	2.5	1.4
14. 7.81	0.9	0.4	0.1	0.4	0.2	0.2
21. 7.81	1.4	0.9	0.8	0.8	0.7	0.6
23. 9.81	-0.2	0.6	0.2	0.5	-0.1	0.3
6.10.81	0.6	0.7	0.7	0.2	1.0	0.5
12.10.81	0.6	0.9	0.5	0.8	0.1	1.0
19.10.81	0.4	0.8	0.4	0.5	1.3	2.2
21.10.81	0.5	0.3	0.7	0.3	0.0	-0.2
28.10.81	0.3	0.6	0.3	1.5	0.1	0.7
4.11.81	-0.4	2.5	-0.4	-0.4	22.7	10.2
11.11.81	1.5	1.2	0.8	1.1	2.7	3.0
18.11.81	0.3	1.0	0.2	0.9	0.8	1.0
25.11.81	0.6	0.5	0.8	0.6	6.0	19.1
2.12.81	0.5	0.6	-0.3	0.6	0.9	-
9.12.81	0.9	0.4	0.1	0.5	1.5	7.7
20. 1.81	0.2	0.5	-0.4	0.3	-0.2	3.8
4. 2.82	0.6	0.0	0.6	0.2	0.5	0.5
11. 2.82	-0.1	-0.3	1.1	0.4	0.5	1.3
17. 2.82	1.5	2.0	0.8	3.3	0.7	2.3
26. 2.82	0.4	0.1	0.3	0.6	0.5	0.4
4. 3.82	0.7	1.2	1.9	1.2	4.0	7.6
7. 3.82	0.2	0.7	0.8	1.1	0.7	1.7
18. 3.82	-0.2	0.0	-0.5	3.9	8.2	34.4
24. 3.82	-0.4	0.2	0.7	0.6	1.4	9.2
1. 4.82	-0.4	0.0	-0.5	0.3	0.2	9.2
7. 4.82	0.0	0.3	-0.4	1.6	2.4	58.9
15. 4.82	-0.5	-0.1	-0.5	0.1	0.3	5.4
22. 4.82	0.8	1.7	0.8	3.5	2.3	4.5
28. 4.82	0.7	4.8	3.1	5.0	3.2	23.3
30. 4.82	0.6	0.6	1.2	0.5	3.9	69.7
7. 5.82	1.3	1.1	3.5	-	4.6	99.5
11. 5.82	0.2	-0.4	0.7	-0.3	2.9	18.7
19. 5.82	0.9	0.8	0.4	1.1	4.9	6.4
26. 5.82	0.3	1.0	1.1	1.7	3.8	5.7